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ADRENAL CORTEX EXTRACT*

A CONSIDERATION OF ITS USE IN VARIOUS TYPES OF ADRENAL INSUFFICIENCY AND RELATED CONDITIONS

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IN COMMON with other endocrine structures, the adrenal glands have attracted considerable interest, particularly in recent years because of the more precise knowledge of the function of the cortex and the availability of potent extracts. In view of the wide scope of investigation in various quarters and the growing literature, it is now possible to chart the trend of such developments. These may be broadly divided into three general groups: (1) The relation of the adrenal cortex to mineral metabolism as noted in the alterations induced by experimental extirpation and the fairly similar changes in Addison's disease; (2) the interrelationship of the adrenals to other endocrine structures and their part in pluriglandular syndromes; (3) certain associated effects and functions not clearly delineated, such as the relation of the cortex to immune processes, vitamin metabolism and similar topics. The first group is so far the most accurately studied though the practical application of this knowledge is restricted to the small group of Addison's disease. The evidence in the latter two groups is still equivocal; the subject matter covers a wide range of clinical problems and unfortunately is a fertile field for vague speculative articles not based upon substantial scientific data. Attracted to these problems in the course of experiments upon the preparation

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of a potent adrenal cortex extract,* we have thought it of interest to epitomize the results of adrenal cortex therapy upon sixty patients who have been considered suitable for inclusion in one or more of the three categories mentioned above. The age periods in this study ranged from infancy to the sixth decade. A few newborn infants who were treated for adrenal hemorrhage syndrome or marasmus are included, though no encouraging results were obtained.

In order to indicate the theoretical basis and limitations of the clinical use of adrenal cortex extract, it is desirable to review briefly the experimental linking of the adrenal cortex to mineral metabolism.

Harrop[1] and his collaborators studied the changes produced in adrenalectomized dogs by the cessation of daily injections of cortical extracts after a maintenance level had first been established. By this method and by the use of nembutal anesthesia, a number of variables were eliminated. The principal changes induced by the cessation of cortical extract were, hemoconcentration with loss of water and salt, retention of blood urea nitrogen, increase of plasma proteins, lessened blood flow and reduced basal oxygen consumption. The loss of fluid was found to be mainly by renal excretion rather than movement into the tissues or gastrointestinal tract. The fall of chlorides was accelerated if salt had been restricted in the diet. It is well established that the movement of the chlorine anion between the blood plasma and cells of the blood and tissue is such that it enables the cell buffers to contribute to the stability of the acid-base equilibrium. The net result of such loss of chlorides with that of sodium and some carbonate is one of compensated alkali deficit. The nitrogen retention which has been found both in experimental and clinical observations has usually been attributed to renal failure consequent upon lack of fluid and fall in blood pressure. However Harrop and others believe that it may be part of a compensatory mechanism to restore osmotic equilibrium after marked loss of electrolytes. Since the approximately 100 m. eq. per liter of chloride ion make up about two-thirds of the ions of the plasma, it is apparent that some readjustment is necessary. Under normal conditions at least, urea is freely permeable through the body membranes and therefore has little value in osmotic equilibrium. Whether this property is reversed in adrenal insufficiency cannot be stated from the evidence so far. Upon resumption of cortical extract injections, they found a restoration of the electrolyte pattern.

Loeb[2] has reported similar changes in adrenalectomized dogs and has indicated that the loss of sodium from the body is an important factor in the development of insufficiency in this animal. He also believes that this loss is by the kidney and not due to vomiting, diarrhea or loss into the tissues. He considers this sodium loss a primary factor due to failure of some regulatory function of the adrenal cortex rather than secondary to loss of fluid or to combinations with excreted acids as in diabetic acidosis. A rise in serum potassium and nonprotein nitrogen was also noted by Loeb. Substantially similar results have been reported by Zwemer and Sullivan[3] in adrenalectomized cats. Numerous clinical observations have indicated that analogous conditions of hemoconcentration and electrolyte dislocations occur in Addison's disease.

*During part of this investigation we used an extract prepared in our own laboratory, later we used a similar extract prepared by the Wilson Laboratories. These extracts are free of adrenalin.

This explanation has not satisfied all observers. Britton and Silvette[4] are among the protagonists of the theory that a disturbance in carbohydrate metabolism produces the manifestations of adrenal insufficiency. They believe that the adrenalectomized animal suffers from a critical fall in glucose and glycogen with consequent collapse and a shift of fluid from the blood to the liver and muscle tissues. Hypoglycemia though a characteristic finding in experimental adrenal insufficiency and frequently in Addison's disease has been usually considered a secondary factor. The hypoglycemia may be severe enough to be a factor in episodes of collapse in patients with Addison's disease, and we have seen typical hypoglycemic shock during a glucose tolerance test in such a patient. The question as to whether the experimental adrenal insufficiency is a counterpart of the clinical disease can be answered in the affirmative with the exception of the absence of pigmentation in the experimental animal; the blood pressure in the adrenalectomized dog is low only in the late stages after dehydration has set in.

The clarification of the chemical disturbances in adrenal insufficiency has naturally indicated the methods by which these changes may be reversed. It has been repeatedly demonstrated that severe manifestations of adrenal insufficiency in both human beings and animals may be prevented for some time by the ingestion of large amounts of sodium chloride, particularly if reinforced with sodium bicarbonate or sodium citrate even without the necessity for also using cortical extract. It is also true that adequate amounts of a potent adrenal cortex extract will assure a normal electrolyte balance without any special efforts to increase the salt content of the diet. At the Mayo Clinic, Wilder[5] and collaborators in a careful study of three patients found that critical symptoms may be delayed if the potassium intake of the diet is kept down to 1.6 gm. or less daily even under sodium chloride restriction. Kendall[6] believes that the hormone has two components, an A fraction responsible for normal muscular activity and a B fraction which enables the combined components to bring the raised blood urea noted in insufficient animals down to normal, presumably by enabling them to retain sodium chloride.

Because of these developments the whole subject has become so well defined that it is now possible to recognize subclinical or mild types of Addison's disease by definite even though slight chemical alterations. This phase in itself has greatly enlarged the clinical outlook at first restricted to the rather small group of fully developed Addison's disease. These individuals go about in a constant state of ill health without episodes severe enough to cause hospitalization and without a clear-cut diagnosis. The following case history is typical of this group:

M. K., a white male aged fifty-seven years, had a gastroenterostomy performed at the age of thirty-eight, for duodenal ulcer. His complaints when first seen in 1933 were weakness, inability to gain weight, anorexia, occasional vomiting and pain to the left of the umbilicus. His pain had not been relieved by a modified Sippy diet, alkalies and a belt for visceroptosis. On examination he had marked pigmentation of the lips and buccal mucosa. Blood pressure was 75/50; x-ray showed a small heart and lung fields suggesting chronic fibroid tuberculosis though the sputum has always been negative for tubercle bacilli. Blood urea nitrogen was 26 mg. and plasma chlorides were 550 mg. per 100 c.c.; serum sodium

varied from 130 to 134 m. eq. per liter. A striking improvement in nutrition, strength and blood pressure was obtained by weekly dosage of only 2 c.c. of adrenal cortex extract. This amount was, however, inadequate to keep the blood chemistry within normal range during the entire interval between injections. The pain has not recurred despite the use of an unrestricted diet.

Another type of case which we have noted with increasing frequency has exhibited manifestations of nutritional deficiency with secondary signs of adrenal failure. Packard and Wechsler[7] have called attention to a syndrome characterized by malnutritional edema, polyneuritis and signs of adrenal insufficiency. However hypotension and pigmentation were not necessarily present. There were in all 25 patients in our series who could be placed in a miscellaneous group of subclinical or mild adrenal insufficiency though only approximately half of them exhibited definite improvement on cortical therapy.

SHOCK

There is no unanimity of opinion upon the mechanism of shock and there is no intention here to go into the controversial aspects of this subject. It is, however, fair to agree with Swingle, Harrop and others that the chemical changes found in experimental adrenal extirpation simulate to a varying degree those found in surgical shock. Indeed Swingle, Pfiffner, Vars, Bott and Parkins[8] have prepared a table of similarities which is quite striking. Among the phenomena which are common to shock and adrenal insufficiency, they list: a decrease in blood volume, blood plasma, rate of blood flow, cardiac output and alkali reserve; an increase in hemoconcentration, blood viscosity, nitrogenous constituents and sensitivity to hemorrhage, trauma and infections. Some of these resemblances have been pointed out by other observers. Atchley[9] believes that a distinction should be made between medical or anhydremic shock and surgical shock. He believes that the former is accompanied by a primary loss of blood volume due to dehydration with recovery on replacement of fluid. It is possible that some deaths in diabetic coma are of this nature. Transudation of fluid does not occur into the tissues in this type. Traumatic shock on the contrary may be dependent upon a loss of capillary tone, interference with tissue circulation and a resultant loss of circulating blood volume; if fluid or plasma is lost as it is in some cases with toxic capillary change, it presumably is lost into the tissues. Therefore the mechanism of adrenal insufficiency fits into the category of anhydremic or medical shock. In other respects the resemblance of adrenal insufficiency to a shocklike state is well borne out. The sensitivity of the adrenalectomized animal to hemorrhage, trauma, toxins, drugs, and the like is quite marked.

In the application of experimental findings to the clinical use of cortical extract in shock, several serious objections must be met. The fact that the extract replaces the lost function in the adrenalectomized animal does not necessarily mean that similar results will occur in hemoconcentration due to a different cause and a different mechanism. On the contrary the hormone may act in the presence of a disturbed physicochemical state of the circulation such as has been outlined above, even if this state has not been primarily produced by loss of adrenal function. Practical experiences seem to support

the latter view and this would make it a valuable adjunct in the prevention and treatment of surgical and other forms of shock. Some of these conditions were fulfilled in the following case, though the ultimate outcome was uninfluenced:

A white female aged sixty-two years, suffering from biliary cirrhosis, adhesions about the gallbladder and ascites was seen twenty-four hours after operation in severe shock. Her blood pressure which was 60/40 eighteen hours after operation had been temporarily raised to 100/50 by intravenous glucose in saline solution though the cell-plasma ratio still indicated hemoconcentration. Two hours after an injection of 2 c.c. of adrenal cortex extract, the blood pressure rose to 140/70 and remained well sustained for another twelve hours. The patient died about forty-eight hours after operation with so called "hepatic toxemia."

In the preparation of patients with thrombopenic purpura for splenectomy we have occasionally observed instances of hemoconcentration and in consequence have used adrenal cortex extract in conjunction with other routine procedures to guard against the possibility of shock. Recent evidence that the susceptibility to shock of individuals with the thymic habitus may be related to adrenal insufficiency, suggests the probable prophylactic value of adrenal cortex extract in such patients. The emphasis placed upon the shock aspects of diabetic coma in the reports by Atchley and also Lande[10] would suggest the use of adrenal cortex extract along with the other recommended measures in these instances. If this is contemplated, it must be remembered that there is an element of danger if hydremia instead of hemoconcentration is present and if the heart and kidneys are not competent to meet the demands of an influx of fluid into the circulation. Two patients with extensive burns were treated with adrenal cortex in conjunction with other standard measures. In one patient, a girl of nine, there appeared to be a favorable effect on dehydration and shock, though she as well as the second patient succumbed after several weeks.

RESPIRATORY METABOLISM

Low basal metabolic rates are quite constantly a feature in well-developed cases of Addison's disease. The experimental analysis of this phenomenon has been thoroughly studied by Swingle and Pfiffner.[11] From the clinical viewpoint it can be said that the effect of cortical hormone upon the metabolism of patients with Addison's disease is not constant. However, Greene, Rowntree, Swingle and Pfiffner[12] found that if the basal metabolic rate of the patient before treatment has been low, the hormone has a tendency to raise it toward normal. This increase is not necessarily commensurate with the increase in weight, strength or vitality of the individual. The analysis of these effects is naturally more involved than in the experimental animal and still more so in pluriglandular syndromes exhibiting dysfunction or failure of the adrenotropic and thyrotropic hormones of the pituitary such as Simmond's cachexia and related disorders. It would appear that the blood cholesterol level would be of aid in estimating the thyroid component in the low metabolic rates of Addison's disease and such pluriglandular syndromes as mentioned above. Occasionally extremely high cholesterol readings have been noted in Addison's disease though Harrop and Weinstein[13] reported normal

figures in these patients even with basal metabolic rates as low as minus 24 per cent. Two of our patients with low basal metabolism though exhibiting different endocrine manifestations did well on adrenal cortex extract after unfavorable response to thyroid extract.

One of these, M. M., a heavily built man aged forty-seven years, complained of asthenia and fatigability; he was found to have hypotension, small areas of pigmentation on the lower lip, a basal metabolic rate of minus 34 per cent and a blood cholesterol of 294 mg. per 100 c.c. though without objective evidence of myxedema. Thyroid extract by mouth was not followed by any improvement and in fact made him more irritable. On weekly injections of 2 c.c. of adrenal cortex extract over a period of three months, the asthenia was definitely improved and the basal metabolic rate six months later was minus 13 per cent. However the systolic blood pressure had increased by only 10 mm. of mercury.

GONADS

Despite the wide acceptance from a clinical and even from a pathologic viewpoint of a connection between adrenal and gonadal function, there is a surprising lack of uniformity in actual animal experiments bearing upon this question. The various hypotheses proposed to explain these results may be found in a comprehensive review by Britton,[14] also in a recent article by Howard and Grollman.[15] The latter attribute the contradictory reports in the literature to impurities in the extract or to spontaneous variability in the experimental animal. They also believe that if alterations in the sexual functions of adrenalectomized rats occur, they merely represent one phase of a general bodily change.

The clinical observations upon this relationship are found in reports which cite improvement in adrenogenital syndromes following surgery upon hyperplastic or neoplastic adrenal tissue. Broster, Gardiner Hill, and Greenfield[16] have performed unilateral adrenalectomy on three patients each representing a different type of this syndrome: pseudohermaphroditism, virilism and Achard-Thiers syndrome (diabetes, obesity, and hirsutism in women). The most definite improvement was in the patient presenting virilism of the adrenal type. Walters, Wilder, and Kepler[17] believe that cortical adenoma or hyperplasia of the adrenal cortex may be suspected in female patients if they present a syndrome characterized particularly by obesity of face, trunk, and abdomen, hirsutism, hypertension, hyperglycemia, and amenorrhea. In their report several patients presenting similar manifestations were found to have basophilic adenomas of the pituitary (Cushing's syndrome). They believe that surgical removal of the adrenal tumor or partial resection of hyperplastic adrenal cortex may be helpful. Syndromes such as described above are considered by some to be hypophyseal in their origin with excess of adrenotropic and gonadotropic hormones. In these types and in the adrenal syndromes of both sexes before puberty such as virilism, pseudohermaphroditism and pubertas precox, Howard and Grollman[15] can find no evidence that the cortical hormone per se is responsible for these changes. Kendall[6] reconciles some of these diverse views by suggesting that the manifestations noted in these patients may be in the nature of dysfunction of the cortical hormone rather than mere overproduction of normal hormone.

The question of replacement therapy in gonadal hypofunction is undoubtedly of greater practical interest. In this category the few positive clinical results we have observed have been found to occur in individuals with concomitant signs suggesting insufficiency of adrenal function. This does not necessarily imply that asthenia or undernutrition was a prerequisite. The most marked stimulation characterized by increased libido and erection occurred in a heavily muscled middle aged male who had hypotension and minor mucous membrane pigmentations. Associated signs of gonadal stimulation along with general bodily vigor and improvement in hypotension were also noted in several young married women. Hartman, Beck, and Thorn,[18] have also described the increased sexual vigor attendant upon bodily and mental improvement after cortical therapy in patients who presented manifestations of functional derangement of the adrenal gland.

Interrelationship among the pituitary, adrenal and gonads are exemplified in such syndromes as Simmond's cachexia. A clearer insight into this endocrine disorder has been gained due to recent experimental observations upon the adrenotropic and gonadotropic hormones of the pituitary. The conclusion of most investigators is that severe pituitary deficiency causes atrophy of the adrenal cortex. In a carefully controlled series of experiments in rats Shumacker and Firor[19] showed that compensatory hypertrophy of the remaining adrenal did not occur if unilateral adrenalectomy was done in hypophysectomized animals. Stunting of growth, inactivity and lowered body temperature resulted from either pituitary or adrenal deprivation. Pituitary transplants were effectual in pituitary deprivation and adrenal cortical extracts for adrenal insufficiency. Pituitary transplants were not effectual if entire adrenal tissue was absent. The atrophy of the reproductive system seemed to be related to the absence of the gonadotropic hormone of the pituitary in both the hypophysectomized and adrenalectomized animals. In the light of such observations and numerous clinical and pathological studies, the mechanism of such syndromes as Simmond's cachexia becomes reasonably clear. The low basal metabolism and blood sugar, the hypoplasia of the circulatory system with hypotension are all apparently related to the adrenal failure. These subjects respond to extracts derived from the pituitary and adrenal cortex. They are apt to react poorly to insulin very much as do cases of Addison's disease. As a matter of fact the use of the adrenal cortex extract is urgently indicated in the late stages of the disease when profound asthenia and prostration indicate a failing if not already absent adrenal function. Evidence in this direction was derived from the data of a woman aged forty-six years* (service of Dr. J. C. Doane) who had presented the complete clinical picture of Simmond's cachexia. At necropsy the adrenals were replaced by unrecognizable fragments of tissue containing some chromaffin framework.

In their incipiency particularly in early life these syndromes may not be well defined and are apt to present trying problems of diagnosis and management. The following patient (the subject of a fuller report by Wohl and Ettelson) is an example of this type.

*The complete details of this case are described by Dr. Doane and Dr. Gouley in a forthcoming report.

J. C., a female, aged sixteen years had noticed failing appetite and strength during the past year. The present weight was 82 pounds which represented a loss of 25 pounds during the same period. There was a cessation of menstruation. Pallor was noted though the blood count showed only a moderate lowering of hemoglobin to 13.5 gm. per 100 c.c. with a normal red cell count. Pulse rate was 44 to 50 per minute, blood pressure was 82/70; x-ray showed hypoplasia of the heart and large vessels, basal metabolic rate was minus 34 per cent with a blood cholesterol of 145 mg. per 100 c.c. Blood sugar 87 mg. per 100 c.c. with a fairly normal glucose tolerance, though highest peak of curve was at 150 mg. She had none of the skin changes of Simmond's disease but showed a slight bluish pigmentation of lips and roof of the mouth. Dietetic management and thyroid medication had no effect. Upon three injections a week of adrenal cortex extract (25 gm. per 1 c.c.) in doses of 1 c.c., she improved in strength, mental outlook and gained 12 pounds in about three months. Blood pressure increased only slightly from an average of 80/60 to 90/70.

Another young female in a similar category with the added feature of allergy was also observed. The patient was an unmarried female aged twenty-four years who though 5 feet 2 inches in height weighed only 80 pounds. She suffered from asthenia and anorexia. In addition bronchial asthma had been present since childhood. There was a sensitivity to milk and wheat proteins verified by intradermal reactions and she was symptomatically sensitive to various meats though not strikingly reactive to skin tests. She showed a general underdevelopment of secondary sex characteristics; blood pressure was 95/55; basal metabolism rate was –20 per cent. She slowly gained 10 pounds in about four months under treatment with cortical extract though not receiving the extract regularly. During this period the asthma was definitely improved. In this regard the recent work of Wolfram and Zwemer[20] is of interest in that it apparently lends some support to the clinical application of cortical hormone in allergic states. In egg albumen anaphylaxis in normal guinea pigs, they found a greater proportion of survivals in those receiving cortical hormone, particularly when given two to six hours before the shocking dose.

VITAMIN METABOLISM

The uncontradicted evidence that the adrenal gland is a rich source of vitamin C has furthered the thought that the adrenal gland (particularly the cortex) was in some way connected with vitamin metabolism. It was found, for example, that changes occurred in some avitaminoses—atrophy in avitaminosis A and hypertrophy in avitaminosis B and C. Some investigations appeared to have established the efficacy of adrenal extracts in the treatment of deficiency due to deprivation of vitamins B and C. The dependence of the contained vitamin C upon dietary factors was noted by Siehrs and Miller[21] who found that ascorbic acid promptly disappeared from the adrenals of guinea pigs on a scorbutic diet and reappeared when adequate amounts of orange juice were added to the diet. Recently Baena[22] found that albino rats could run longer in an exercise apparatus if treated with adrenal cortex extract and ascorbic acid than if injected with cortical extract only. The whole situation has been critically reviewed by Grollman and Firor.[23] They found that under properly controlled conditions a purified cortical extract had no protective effect in animals suffering from scurvy or vitamin B_1 deprivation. Likewise ascorbic acid had no ameliorating effect upon the course of adrenal insufficiency in rats.

Though these results modify pre-existing views on the subject, the logical presentation of the authors and the controlled conditions of the study lend weight to the supposition that the connection between the adrenals and vitamins is not a direct one. On the other hand the absolute exclusion of the vitamin under investigation does not pertain to most human deficiencies. The

possibility that under such partial deprivation the cortical hormone or some unknown substance contained in the extracts of the gland may condition the amount of vitamin necessary or aid in its utilization, as suggested by Lockwood and Hartman,[24] cannot therefore be altogether ignored. There is some resemblance also in the susceptibility to infections and intoxications exhibited by adrenalectomized rats and the similar susceptibility in clinical and experimental scurvy. Comparable thoughts concerning the interrelations of hormones and vitamins have been recently expressed in an editorial of the Journal of the American Medical Association.[25] As an incidental observation we have repeatedly noted in adrenalectomized rats a soreness of the inner canthus of the eye (not xerophthalmia) on diets apparently ample in vitamin B complex for normal rats. The only direct effect which we have noted clinically was in the patient described under the heading of postoperative edema. In this patient a definite pellagrous like eruption disappeared within a few days after cortical hormone administration and before any other change in diet or therapeutics had been instituted. Whether a similar effect could be obtained with the refined hormone which we are now using, it is not possible to state at this time.

POSTOPERATIVE NUTRITIONAL EDEMA

Occasionally edema which is not related to cardiac or renal disease appears in postoperative patients. As pointed out by Jones and Eaton,[26] the edema appears to belong to the category of nutritional deficiency and is usually accompanied by hypoproteinemia. In their series it appeared after surgical operations upon the gastrointestinal tract and in patients with definite evidence of sub-nutrition. Usually large quantities of fluid had been given parenterally after operation in the form of salt solution. We have had occasion to note this syndrome as a postoperative complication and in each instance there has been a definite concomitant state of nutritional deficiency. An editorial in the Journal of the American Medical Association[27] emphasizes the probable pathologic shift of fluids and electrolytes in such conditions. The possible therapeutic benefit of restoring such shift by adrenal cortex extract was suggested by the events in the following case history:

H. I. R., female, aged forty-two years (service of Dr. R. Boyer and Dr. J. C. Doane), following a long period of Sippy diet was operated upon. The laparotomy revealed calculous cholecystitis and a cholecystectomy was performed. For several weeks thereafter she remained in a state of profound asthenia and with a blood pressure as low as 60/50. Intravenous injections of glucose and saline solutions were used but an increasing edema was noted. A scaly dermatitis of the neck and arms somewhat resembling a pellagrous eruption appeared. The blood urea nitrogen about one month after operation was 116 mg. per 100 c.c.; blood sugar, 69 mg.; chlorides 420 mg. per 100 c.c. (whole blood); the blood count revealed a moderate secondary anemia. Unfortunately serum proteins were not done.

At this time adrenal cortex extract was started in daily dosage of 2 c.c. (1 c.c. = 30 gm. of whole gland). Within several hours after the first injection the blood pressure was 80/60 but fell back again to 60/50 the next day. Subsequently the systolic blood pressure varied between 90 and 100 mm. of mercury. The edema and the pellagrous like eruption disappeared and the patient was strong enough to be discharged about one week after the inception of adrenal cortex injections. She succumbed later at home to a subphrenic suppuration. The significant data while under treatment are summarized in Table I.

TABLE I

DATE	ADRENAL CORTEX EXTRACT	B.P. MM. OF HG.	UREA N. MG.
12-2	2 c.c.	60/50	116
2 hours later		80/60	
12-3	2 c.c.	60/50	
12-4	2 c.c.	90/70	100
12-5	2 c.c.	100/60	61
12-6	2 c.c.		
12-7	2 c.c.	100/65	33
12-7	2 c.c.	100/60	29

BLOOD

There are comparatively few reports regarding the hematologic features of adrenal insufficiency. Corey and Britton[28] noted that in cats the red cells after adrenalectomy increased from an average of 9,800,000 to 15 million per c. mm. In the light of the observations already mentioned, one would be inclined to relate this change to hemoconcentration with resultant higher cell-plasma ratio. However there was also noted a fall in the leucocyte count mainly affecting the neutrophiles which could be raised again by the injection of cortical extract. It has been occasionally suggested that the use of adrenal cortex extract may be effective in agranulocytic syndromes. Fitz-Hugh[29] has recently commented on this possibility. We had occasion to study the effects of this substance in two young women and one young man during a granulopenic phase in the course of infectious mononucleosis. In one patient, a nurse, membranous lesions in the mouth promptly disappeared though no demonstrable effect on the blood picture was noted. In the second patient small aphthous ulcers also cleared up, asthenia improved, but the abnormal blood picture persisted for many weeks thereafter. Since the administration of the extract in each case was limited to a few injections it is not possible to estimate the effect of long continued administration. The possibility is suggested that the hormone may complement or condition some function ordinarily assumed by granular leucocytes and in the absence of which the necrotic lesions so characteristic of agranulocytic processes are noted. This thought seems to be borne out by the work of Szent-Györgyi[30] who believes that the adrenal cortex is connected in some way with the oxidizing mechanism of the peroxidase system.

INFECTION

The susceptibility of adrenalectomized animals to infections and intoxications has led experimenters to investigate the possibility that adrenal function is an essential link in the immunity mechanism. Hartman and Scott,[31] using adrenalectomized rats, believe they have demonstrated that adrenal cortex extract exerted a definite protective effect against the chronic intoxication produced by suspensions of dead staphylococci and the acute intoxication induced by dead typhoid bacilli. Marmorston-Gottesman and Perla[32] found that bilateral adrenalectomy in Wistar strain rats lowered the natural resistance to infections with *Bartonella muris*. However the acquired immunity due to a previous infection was not altered by a later adrenalectomy.

We have verified the observations of Hartman and Scott with respect to the acute intoxication produced by dead typhoid bacilli (Fig. 1). In addition we have found that adrenalectomized rats are not only incapable of withstanding the large doses of dead typhoid bacilli used in these experiments but that they also succumb during attempts at immunization with repeated graded doses of vaccine, in contrast to the survival of adrenalectomized rats treated with adrenal cortex injections. There is an obvious fallacy in applying these observations to clinical infections. If the whole situation is merely the protective or replacement effect of the hormone in an animal thrown into insufficiency, then there is no true counterpart to the ordinary course of events

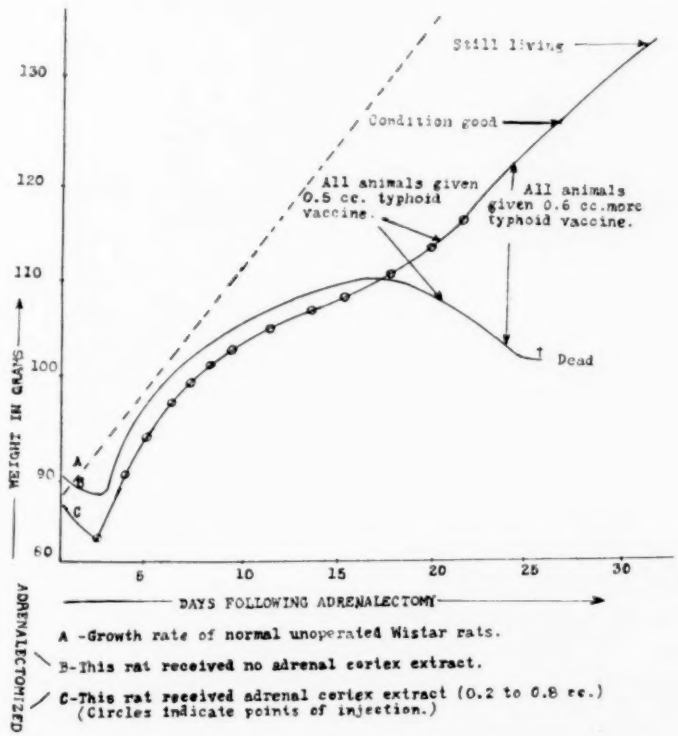

Fig. 1.—Protective effect of adrenal cortex extract against typhoid vaccine intoxication in adrenalectomized rats.

in a clinical infection. On the contrary if adrenal insufficiency could be shown to occur in severe or protracted infections, there would be some logic in the application of these experimental observations to the practical therapy of human infections.

There are pathologic observations which suggest this possibility. Dietrich[33] has described edema of the adrenal cortex in protracted infections; corresponding to the severity of the infection there may be noted degenerative changes in the lipoids of the outer layers of the fasicular zone, the glomerular zone and to a lesser extent, the reticular layer. Aschoff[34] states that these changes are particularly noteworthy in diphtheria, scarlet fever and septic infections particularly those due to streptococci. Fig. 2 illustrates such a case

J. C., a female, aged sixteen years had noticed failing appetite and strength during the past year. The present weight was 82 pounds which represented a loss of 25 pounds during the same period. There was a cessation of menstruation. Pallor was noted though the blood count showed only a moderate lowering of hemoglobin to 13.5 gm. per 100 c.c. with a normal red cell count. Pulse rate was 44 to 50 per minute, blood pressure was 82/70; x-ray showed hypoplasia of the heart and large vessels, basal metabolic rate was minus 34 per cent with a blood cholesterol of 145 mg. per 100 c.c. Blood sugar 87 mg. per 100 c.c. with a fairly normal glucose tolerance, though highest peak of curve was at 150 mg. She had none of the skin changes of Simmond's disease but showed a slight bluish pigmentation of lips and roof of the mouth. Dietetic management and thyroid medication had no effect. Upon three injections a week of adrenal cortex extract (25 gm. per 1 c.c.) in doses of 1 c.c., she improved in strength, mental outlook and gained 12 pounds in about three months. Blood pressure increased only slightly from an average of 80/60 to 90/70.

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The uncontradicted evidence that the adrenal gland is a rich source of vitamin C has furthered the thought that the adrenal gland (particularly the cortex) was in some way connected with vitamin metabolism. It was found, for example, that changes occurred in some avitaminoses—atrophy in avitaminosis A and hypertrophy in avitaminosis B and C. Some investigations appeared to have established the efficacy of adrenal extracts in the treatment of deficiency due to deprivation of vitamins B and C. The dependence of the contained vitamin C upon dietary factors was noted by Siehrs and Miller[21] who found that ascorbic acid promptly disappeared from the adrenals of guinea pigs on a scorbutic diet and reappeared when adequate amounts of orange juice were added to the diet. Recently Baena[22] found that albino rats could run longer in an exercise apparatus if treated with adrenal cortex extract and ascorbic acid than if injected with cortical extract only. The whole situation has been critically reviewed by Grollman and Firor.[23] They found that under properly controlled conditions a purified cortical extract had no protective effect in animals suffering from scurvy or vitamin B_1 deprivation. Likewise ascorbic acid had no ameliorating effect upon the course of adrenal insufficiency in rats.

Though these results modify pre-existing views on the subject, the logical presentation of the authors and the controlled conditions of the study lend weight to the supposition that the connection between the adrenals and vitamins is not a direct one. On the other hand the absolute exclusion of the vitamin under investigation does not pertain to most human deficiencies. The

possibility that under such partial deprivation the cortical hormone or some unknown substance contained in the extracts of the gland may condition the amount of vitamin necessary or aid in its utilization, as suggested by Lockwood and Hartman,[24] cannot therefore be altogether ignored. There is some resemblance also in the susceptibility to infections and intoxications exhibited by adrenalectomized rats and the similar susceptibility in clinical and experimental scurvy. Comparable thoughts concerning the interrelations of hormones and vitamins have been recently expressed in an editorial of the Journal of the American Medical Association.[25] As an incidental observation we have repeatedly noted in adrenalectomized rats a soreness of the inner canthus of the eye (not xerophthalmia) on diets apparently ample in vitamin B complex for normal rats. The only direct effect which we have noted clinically was in the patient described under the heading of postoperative edema. In this patient a definite pellagrous like eruption disappeared within a few days after cortical hormone administration and before any other change in diet or therapeutics had been instituted. Whether a similar effect could be obtained with the refined hormone which we are now using, it is not possible to state at this time.

POSTOPERATIVE NUTRITIONAL EDEMA

Occasionally edema which is not related to cardiac or renal disease appears in postoperative patients. As pointed out by Jones and Eaton,[26] the edema appears to belong to the category of nutritional deficiency and is usually accompanied by hypoproteinemia. In their series it appeared after surgical operations upon the gastrointestinal tract and in patients with definite evidence of sub-nutrition. Usually large quantities of fluid had been given parenterally after operation in the form of salt solution. We have had occasion to note this syndrome as a postoperative complication and in each instance there has been a definite concomitant state of nutritional deficiency. An editorial in the Journal of the American Medical Association[27] emphasizes the probable pathologic shift of fluids and electrolytes in such conditions. The possible therapeutic benefit of restoring such shift by adrenal cortex extract was suggested by the events in the following case history:

H. I. R., female, aged forty-two years (service of Dr. R. Boyer and Dr. J. C. Doane), following a long period of Sippy diet was operated upon. The laparotomy revealed calculous cholecystitis and a cholecystectomy was performed. For several weeks thereafter she remained in a state of profound asthenia and with a blood pressure as low as 60/50. Intravenous injections of glucose and saline solutions were used but an increasing edema was noted. A scaly dermatitis of the neck and arms somewhat resembling a pellagrous eruption appeared. The blood urea nitrogen about one month after operation was 116 mg. per 100 c.c.; blood sugar, 69 mg.; chlorides 420 mg. per 100 c.c. (whole blood); the blood count revealed a moderate secondary anemia. Unfortunately serum proteins were not done.

At this time adrenal cortex extract was started in daily dosage of 2 c.c. (1 c.c. = 30 gm. of whole gland). Within several hours after the first injection the blood pressure was 80/60 but fell back again to 60/50 the next day. Subsequently the systolic blood pressure varied between 90 and 100 mm. of mercury. The edema and the pellagrous like eruption disappeared and the patient was strong enough to be discharged about one week after the inception of adrenal cortex injections. She succumbed later at home to a subphrenic suppuration. The significant data while under treatment are summarized in Table I.

TABLE I

DATE	ADRENAL CORTEX EXTRACT	B.P. MM. OF HG.	UREA N. MG.
12-2	2 c.c.	60/50	116
2 hours later		80/60	
12-3	2 c.c.	60/50	
12-4	2 c.c.	90/70	100
12-5	2 c.c.	100/60	61
12-6	2 c.c.		
12-7	2 c.c.	100/65	33
12-7	2 c.c.	100/60	29

BLOOD

There are comparatively few reports regarding the hematologic features of adrenal insufficiency. Corey and Britton[28] noted that in cats the red cells after adrenalectomy increased from an average of 9,800,000 to 15 million per c. mm. In the light of the observations already mentioned, one would be inclined to relate this change to hemoconcentration with resultant higher cell-plasma ratio. However there was also noted a fall in the leucocyte count mainly affecting the neutrophiles which could be raised again by the injection of cortical extract. It has been occasionally suggested that the use of adrenal cortex extract may be effective in agranulocytic syndromes. Fitz-Hugh[29] has recently commented on this possibility. We had occasion to study the effects of this substance in two young women and one young man during a granulopenic phase in the course of infectious mononucleosis. In one patient, a nurse, membranous lesions in the mouth promptly disappeared though no demonstrable effect on the blood picture was noted. In the second patient small aphthous ulcers also cleared up, asthenia improved, but the abnormal blood picture persisted for many weeks thereafter. Since the administration of the extract in each case was limited to a few injections it is not possible to estimate the effect of long continued administration. The possibility is suggested that the hormone may complement or condition some function ordinarily assumed by granular leucocytes and in the absence of which the necrotic lesions so characteristic of agranulocytic processes are noted. This thought seems to be borne out by the work of Szent-Györgyi[30] who believes that the adrenal cortex is connected in some way with the oxidizing mechanism of the peroxidase system.

INFECTION

The susceptibility of adrenalectomized animals to infections and intoxications has led experimenters to investigate the possibility that adrenal function is an essential link in the immunity mechanism. Hartman and Scott,[31] using adrenalectomized rats, believe they have demonstrated that adrenal cortex extract exerted a definite protective effect against the chronic intoxication produced by suspensions of dead staphylococci and the acute intoxication induced by dead typhoid bacilli. Marmorston-Gottesman and Perla[32] found that bilateral adrenalectomy in Wistar strain rats lowered the natural resistance to infections with *Bartonella muris*. However the acquired immunity due to a previous infection was not altered by a later adrenalectomy.

We have verified the observations of Hartman and Scott with respect to the acute intoxication produced by dead typhoid bacilli (Fig. 1). In addition we have found that adrenalectomized rats are not only incapable of withstanding the large doses of dead typhoid bacilli used in these experiments but that they also succumb during attempts at immunization with repeated graded doses of vaccine, in contrast to the survival of adrenalectomized rats treated with adrenal cortex injections. There is an obvious fallacy in applying these observations to clinical infections. If the whole situation is merely the protective or replacement effect of the hormone in an animal thrown into insufficiency, then there is no true counterpart to the ordinary course of events

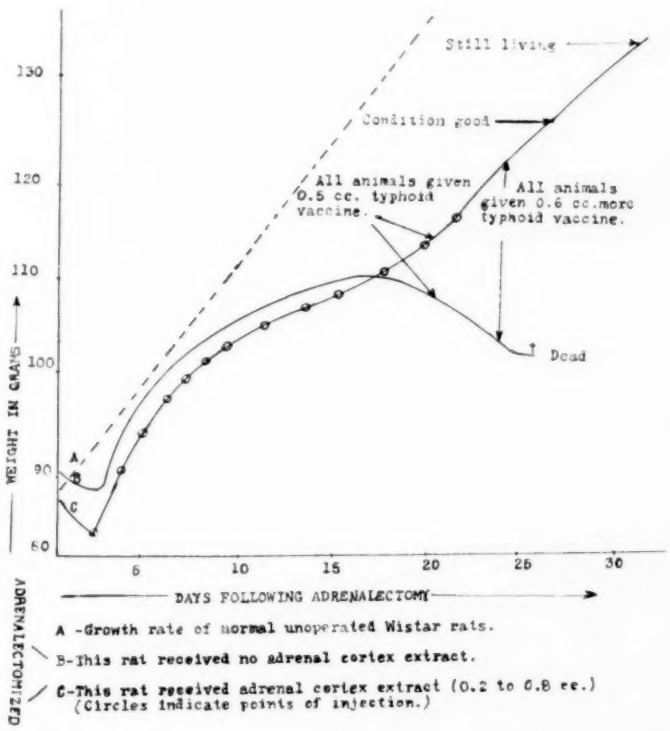

Fig. 1.—Protective effect of adrenal cortex extract against typhoid vaccine intoxication in adrenalectomized rats.

in a clinical infection. On the contrary if adrenal insufficiency could be shown to occur in severe or protracted infections, there would be some logic in the application of these experimental observations to the practical therapy of human infections.

There are pathologic observations which suggest this possibility. Dietrich[33] has described edema of the adrenal cortex in protracted infections; corresponding to the severity of the infection there may be noted degenerative changes in the lipoids of the outer layers of the fasicular zone, the glomerular zone and to a lesser extent, the reticular layer. Aschoff[34] states that these changes are particularly noteworthy in diphtheria, scarlet fever and septic infections particularly those due to streptococci. Fig. 2 illustrates such a case

from our own series. Robbins[35] reported the case of a young woman who developed marked signs of adrenal insufficiency during a streptococcic infection of the throat. The prompt amelioration of these manifestations after the use of adrenal cortex extract (Hartman) seemed to be more than coincidental. If the protection exhibited by the hormone could be shown to be due to actual reinforcement of the immune mechanism, this might also lend credibility to reports upon the favorable effect of adrenal cortex extract in infections. Whitehead and Smith[36] have reported upon the use of the extract

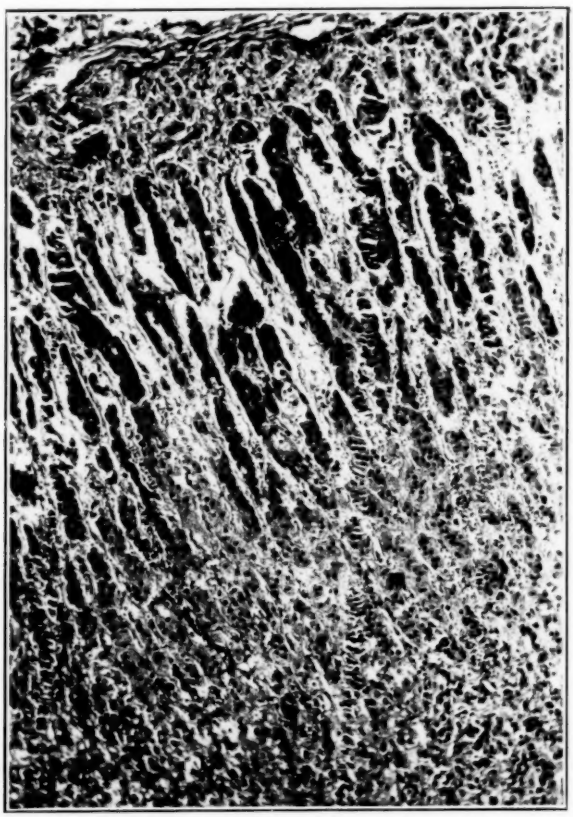

Fig. 2.—Widespread vacuolization and destruction of the fascicular zone of the adrenals in a young woman suffering from a long-standing streptococcic infection.

in the treatment of five patients with various infections, one of which was typhoid fever. He believed that some benefit was detected. Our own experience in this category has been limited to a few patients suffering with staphylococcus aureus bacteremia and in several patients with infectious (atrophic) arthritis. In the latter group one patient particularly illustrates the necessity for selecting the appropriate type of case.

I. M., male, aged fifty-two years, suffered with a fairly generalized and disabling atrophic arthritis which appeared subsequent to gallbladder surgery for calculous cholecystitis. There was a swarthy pigmentation which was general and not particularly characteristic of Addison's disease. However, anorexia, malnutrition, a blood pressure of 70/55 and the pres-

ence of healed fibroid pulmonary tuberculosis, suggested the necessity for adrenal cortex administration especially since dietetic and other measures including vaccines gave no improvement. On injections of 1 c.c. (30 gm. whole gland) twice a week for eight weeks, appetite improved, there was a gain in weight of 12 pounds, and the blood pressure remained about 110/70 at discharge from the hospital. With weekly injections at home the patient after several more months reached a weight of 140 pounds, representing a total gain of 20 pounds, blood pressure 125/80 and a surprising improvement in the arthritis.

Comment.—Though there was present an apparent indication for the use of the hormone in this patient and possibly in others we have seen, its indiscriminate use in unselected cases is obviously futile. Since clinical observations are fraught with the usual uncertainty common to uncontrolled conditions, we must await further confirmation of results which so far are merely suggestive.

SUMMARY

Wider use of adrenal cortex therapy may be expected due to recognition of mild or subclinical variants of Addison's disease especially if significant changes in blood electrolyte pattern can be demonstrated. Similar indications are found in certain pluriglandular disturbances such as Simmond's cachexia and other disorders involving adrenal interrelations with pituitary and gonads.

The use of adrenal cortex in other conditions not so clearly demarcated is discussed. These include particularly shock, vitamin deficiency syndromes and infections. Emphasis is placed upon individualization by selection of proper material and the presence of changes suggesting the need of adrenal cortical hormone.

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STUDIES ON YEASTLIKE FUNGI ISOLATED FROM PULMONARY DISEASE (BRONCHOMONILIAS)*

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INTRODUCTION

IT IS now generally recognized that a number of diseases are caused by fungi. Recently the yeastlike fungi grouped under the name of monilia are stimulating increasing interest as the etiologic factor in various diseases. Progress in their study, however, has been hampered by the lack of dependable methods of classifying them into their respective species.

HISTORICAL

The first mention of the name monilia in the literature was made by Persoon (1797 and 1801) who applied it to a group of twelve fungi which he placed into this genus. The next mention was made in 1839 when Langenbeck isolated the organism which we now recognize as *Monilia albicans* from a case of thrush. Since then, the name monilia has occurred in the literature from time to time; frequently under other names, such as, saccharomyces, parasaccharomyces, and mycoterula (Gay, 1935). Monilias have been isolated from vaginal secretions, skin infections, bone infections, abscesses, gastrointestinal tract, respiratory tract, maxillary sinuses, thrush and sprue. Ashford (1917) has reported the isolation of a certain monilia from one hundred cases of sprue which he has called *Monilia psilosis*.

Bronchomoniliasis is the name given to infections of the respiratory tract caused by any of the organisms of the genus Monilia. The first case of this kind was reported by Castellani in 1905 in Ceylon, India. The patient was employed by a Ceylon firm as a "tea taster" and the disease was referred to as "the tea taster's cough." In the United States the first case reported was one by Boggs and Pincoffs (1915) of Baltimore. Since then, many other cases have been reported (Warr, 1931; Warnock, 1936; Haythorn and associates, 1932; Baskt and associates, 1934; Tanner and Tanner, 1927). The largest group was reported by Flinn, Flinn and Flinn (1935) of Prescott, Arizona, who described nine cases. Recently Ikeda (1936) reported the finding of monilia in specially stained sections of fatal respiratory diseases and in which the organism had been missed in previous examinations.

Classification of these yeastlike organisms (monilia) into species is a rather difficult problem because the methods which are available are not entirely reliable. Carbohydrate fermentation and agglutination tests have been used most extensively. Both have, however, been found unsatisfactory (Brenhan, 1931; Wachowiak and associates, 1934; Lamb and Lamb, 1933, 1935; Hines, 1924).

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Organisms which may ferment certain carbohydrates at one time may lose, or gain, this ability temporarily or permanently. Agglutination tests have been found unreliable because the organisms sometimes aggregate spontaneously, probably because of their large size.

Stone and Garrod (1931) studied precipitin tests with monilias using saline heat extracted antigens. They believed that the results obtained in these precipitin tests were consistent. More recently, Lamb and Lamb (1933 and 1935) offered a method for precipitin tests using a carbohydrate fraction of the organism as the antigen. The results of these tests appeared to be quite specific and stable. They have also presented a carbohydrate utilization test employing seven carbohydrates, i.e., dextrose, levulose, maltose, sucrose, xylose, galactose, and inulin. This method also appears to be quite reliable.

My interest in the study of yeastlike organisms (monilia) began in 1933 in Prescott, Arizona, when a fatal case came under the care of Flinn, Flinn, and Flinn with whom I am associated. Monilia had been isolated and identified morphologically and culturally from the patient's sputum previous to his death. At autopsy yeastlike organisms were isolated from the brain and the apices and bases of both lungs. Attempts to classify these organisms into their species were based entirely upon acid and gas production from carbohydrates according to Castellani (1928) and according to him were found to be *M. pinoyi*. Upon reviewing the literature on this subject, it was learned as indicated above that this is not a reliable method since the ability to ferment carbohydrates is a variable one.

OBJECT AND SCOPE

This work was undertaken with the hope of finding a more rapid and reliable method of classifying monilias. Certain strains of monilia have been tested repeatedly (1933, 1934, 1935, and 1937) for their constancy of behavior towards carbohydrates. This work includes the carbohydrate utilization tests, as suggested by Lamb and Lamb (1933, 1935) and precipitin tests with carbohydrate fraction antigens. Agglutination tests were also studied.

METHODS

1. *Isolation of the Organisms from Sputum.*—The following method was used in isolating the organisms from the sputums of suspected cases of bronchomoniliasis.

The patient was given a sterile jar, a mouth wash, and a bottle of sterile water. The mouth wash was a very dilute solution of bichloride of mercury. The patient was instructed to sterilize his mouth and throat with this solution, then to thoroughly rinse his mouth and throat with the water and expectorate any coughed up sputum directly into the sterile jar. If possible the morning sputum was obtained.

The isolation of the organisms in the laboratory was accomplished in two ways: (1) White "flecks" in the sputum were picked out and inoculated on Sabouraud's agar slants or plates. (2) The sputum was mixed with an equal volume of 70 per cent alcohol, and allowed to stand for one to one and one-half hours. Sabouraud's dextrose agar slants or plates were then heavily inoculated with the sterilized sputum.

Both methods yielded equally good results. A visible growth of the organism could be expected in from one to three days. To obtain pure cultures, a saline suspension of a characteristic colony was made; this was streaked over Sabouraud's agar plates. On these plates, the characteristic round, smooth, raised and pasty colonies can be easily distinguished from the contaminators, which are usually pigmented staphylococci. A morphologic and cultural study of eleven organisms from known pulmonary diseases and from two probable cases (Nos. 19 and 21) will be found in Figs. 1 to 4 and in Table I. Organisms numbers 7, 8, 9, 14, 15, and 22 were isolated from 178 consecutive sputum specimens from cases of pulmonary diseases.

2. *Isolation of Monilias from Normal Throats.*—Cultures were made from 100 students at Stanford University in this experiment. The throat of each student was swabbed with sterile cotton and streaked on Sabouraud's agar. The plates were incubated at 37° C. for several days and then examined for yeastlike organisms. Table I shows the morphologic and cultural characteristics of the three species of the organisms obtained in this experiment. Their numbers are 10, 11, and 12.

3. *Carbohydrate Fermentation and Carbohydrate Utilization Tests.*—A. For regular carbohydrate fermentation tests, Durham fermentation tubes were prepared, using a 1 per cent concentration of the carbohydrate (c.p.) in meat infusion broth. The tubes were inoculated from a twenty-four-hour culture growing on Sabouraud's agar, and incubated at 37° C. They were incubated for thirty days or until acid and gas appeared. The following carbohydrates were used: dextrose, levulose, maltose, d-galactose, sucrose, mannite, lactose, dextrin, rhamnose, arabinose, adinite, inulin, sorbite, starch, glycerine, dulcite, insitol, salicin, xylose, raffinose, and erythrite. In the dextrin broth the fungi developed extensive mycelia. The results of the tests when the carbohydrate was attacked are found in Tables II, III, IV, and V. All tests were made in duplicate and stained smears were made on all positives to rule out contamination. If any disagreement was found, the test was repeated.

B. Carbohydrate utilization tests were carried out with dextrose, levulose, maltose, galactose, zylose, sucrose, and inulin. Tubes containing 1 per cent of the individual carbohydrates were inoculated as indicated under A above and incubated for thirty days at 37° C. At the end of the thirty-day period, eight to ten drops of each of the media were tested for the utilization of the carbohydrates with Benedict's qualitative solution. Sucrose and inulin were first hydrolyzed with an equal number of drops of dilute hydrochloric acid before testing with Benedict's solution. The results are recorded in Tables IV and V, utilization being indicated by ⊕ and +.

4. *Preparation of Precipitating and Agglutinating Serums.*—Antiserums were prepared by injecting rabbits with heavy saline suspensions of the organisms. Five heavy loops from a twenty-four-hour culture of the organism on Sabouraud's agar were added to 10 c.c. of saline with 0.5 per cent phenol and incubated for four or five days at 37° C. to kill the organisms. The injections were made in three series of three doses at weekly intervals. On the first day 1 c.c. was injected, on the second day 2 c.c., and on the third day 5 c.c. Six days

TABLE I

CULTURAL AND MORPHOLOGIC STUDIES

SOURCE	STRAIN NO.	GRAM REACTION	ODOR	PLAIN BROTH	AGAR	LEAD ACETATE	RATE OF GROWTH	LITMUS MILK	GELATIN	PATHOGENICITY	GIANT COLONIES	YEARS STUDIED
Sputum	1	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	1933 to 1937
Rabbit 1	1A	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	
Brain	1B	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	
Left lung	1C	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	
Right lung	1D	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	
Sputum	2	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	
Rabbit 2	2A	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	
Sputum	3	+	Yeasty	Sediment	White pasty	–	Slow	–	Inverted Pine Tree	Rabbit	Slow	1934 to 1937
Sputum	4	+	Yeasty	Sediment	White pasty	–	Rapid	–	Inverted Pine Tree	Rabbit	+	1934 to 1937
Sputum	5	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	1934 to 1937
Sputum	7	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	1935 to 1937
Sputum	8	+	Yeasty	Sediment	White pasty	–	Rapid	–*	Inverted Pine Tree	Rabbit	+	1935 to 1937
Sputum	9	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	1935 to 1937
Normal throat	10	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	1935 to 1937
Normal throat	11	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	1935 to 1937
Normal throat	12	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	1935 to 1937
Sputum (?)	13	+	Yeasty	Sediment	White pasty	–	Medium	–*	Inverted Pine Tree	0	+	1936 to 1937
Sputum	14	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	1936 to 1937
Sputum	15	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	1936 to 1937

\+ Indicates positive.
– Indicates negative.
0 Indicates not done.
* Indicates slightly alkaline (1937).

TABLE I—CONT'D

SOURCE	STRAIN NO.	GRAM REACTION	ODOR	PLAIN BROTH	AGAR	LEAD ACETATE	RATE OF GROWTH	LITMUS MILK	GELATIN	PATHOGENICITY	GIANT COLONIES	YEARS STUDIED
Thrush	16	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	1936 to 1937
Mouth	17	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	1936 to 1937
Mouth	18	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	1936 to 1937
Another Lab.	19	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	1936 to 1937
Mouth	20	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	1936 to
Another Lab.	21	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	0	+	1936 to
Sputum	22	+	Yeasty	Sediment	White pasty	–	Medium	–	Inverted Pine Tree	Rabbit	+	1936 to 1937

TABLE II

1933 BIOCHEMIC REACTIONS

SOURCE	STRAIN NUMBER	DEXTROSE	LEVULOSE	MALTOSE	D-GALACTOSE
Sputum	1	⊕	⊕	⊕	±
Rabbit 1	1A	⊕	⊕	⊕	±
Brain	1B	⊕	⊕	⊕	±
Left lung	1C	⊕	⊕	⊕	±
Right lung	1D	⊕	⊕	⊕	±
Sputum	2	⊕	⊕	⊕	±
Rabbit 2	2A	⊕	⊕	⊕	±
Sputum and mouth	6	⊕	⊕	⊕	±

⊕ Indicates acid with gas. + Indicates acid. – Indicates no acid or gas.

TABLE III

1934 BIOCHEMIC REACTIONS

SOURCE	STRAIN NUMBER	DEXTROSE	LEVULOSE	MALTOSE	D-GALACTOSE
Sputum	1	⊕	⊕	⊕	+
Rabbit 1	1A	⊕	⊕	⊕	+
Brain	1B	⊕	⊕	⊕	±
Left lung	1C	⊕	⊕	⊕	+
Right lung	1D	⊕	⊕	⊕	+
Sputum	2	⊕	⊕	⊕	+
Rabbit 2	2A	⊕	⊕	⊕	+
Mouth and sputum	6	⊕	⊕	⊕	+
Sputum	3	⊕	+	⊕	±
Sputum	4	⊕	⊕	+	±
Sputum	5	⊕	+	⊕	±

⊕ Indicates acid with gas. + Indicates acid. – Indicates no acid or gas.

after the last dose, the rabbits were tested. If satisfactory titers of precipitins and agglutinins were present the rabbits were bled.

5. *The Precipitin Test.*—A. *Preparation of Antigen:* Each organism was cultivated on Sabouraud's agar in Petri dishes heavily inoculated for forty-eight hours. The growth was scraped off with a sterile glass slide and emulsified in 10 c.c. of sterile saline. Five-tenths cubic centimeter normal hydrochloric acid was added and allowed to set for twelve hours with occasional shaking. Thereafter the mixture was placed in a water-bath at 100° C. for fifteen minutes. It was then centrifuged and the supernatant liquid drawn off. This was neutralized with normal sodium hydroxide and centrifuged again. The supernatant liquid, which served as the antigen, was then carefully drawn off. Benedict's qualitative solution indicated the presence of reducing sugars. The pH during extraction was 4.8. The antigens showed an average nitrogen content of 5.39 mg. per c.c.

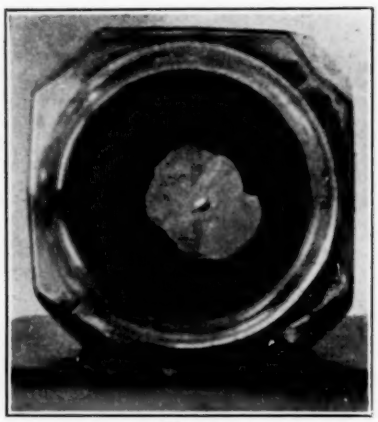

Fig. 1.

Fig. 2.

Fig. 1.—Two-weeks-old growth of *Monilia albicans (M. pinoyi castellani)* on Sabouraud's dextrose agar.

Fig. 2.—One-month-old growth of *Monilia albicans* on Sabouraud's dextrose agar.

B. *Precipitin Test:* The following antigen dilutions were employed: $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{1200}$, $\frac{1}{1400}$, and $\frac{1}{1800}$. Three-tenths cubic centimeter of each of the dilutions was carefully layered over 0.3 c.c. of the serum. Controls with a normal rabbit serum were used. The tests were read for precipitin ring formation after five minutes and were then incubated at 37° C. for one hour, after which they were read again and placed in the icebox overnight. Final readings were made in the morning. The results are given in Table VI.

C. *Precipitin Absorption Test:* To five cubic centimeters of each of the precipitating serums was added 5 c.c. of a heavy suspension of the organisms, and the mixture was shaken for one-half hour at room temperature. The individual mixtures were then placed in the incubator for one hour at 37° C. with occasional shaking. Following this, they were stored in the ice box overnight, centrifuged and the supernatant fluid constituted the absorbed serum. Each absorbed serum was tested against its homologous antigen in one-half dilution for residual precipitating antibody. If complete absorption had taken place, the

absorbed serums were run against all of the antigens in a 1:2 dilution. If antibodies remained the absorption was repeated. Each of the twenty-two strains studied gave no precipitate when tested with each of the nine absorbed serums.

6. *Agglutination Tests.*—A. *Preparation of Fungus Suspensions:* To eighteen-hour cultures of the organisms on Sabouraud's agar slants, 10 c.c. of saline were added. The growth was scraped off with an inoculating loop and well suspended. About 8 c.c. of each suspension were placed in a sterile test tube. The suspensions were well shaken for one half hour. Five-tenths cubic centimeter of each of the following dilutions of the antiserums was prepared: $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{1200}$, $\frac{1}{1400}$, $\frac{1}{1800}$. Five-tenths cubic centimeter of the organism suspension was added to each serum dilution. These were mixed well and incubated for two hours at 37° C. The tests were read and incubated overnight in the ice box. Final readings were made in the morning. The results of these agglutination tests are given in Table VI.

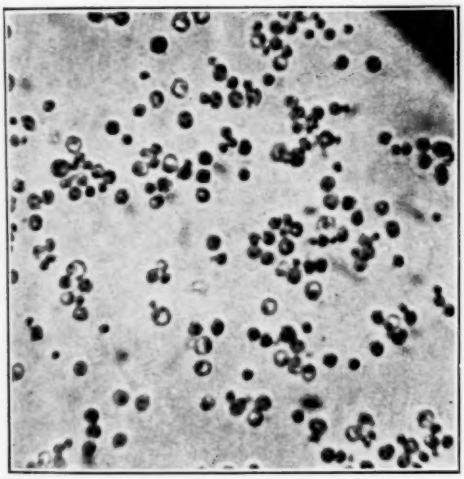

Fig. 3.

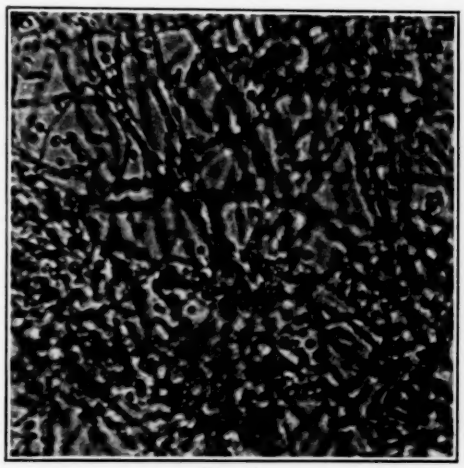

Fig. 4.

Fig. 3.—A twenty-four-hour growth in dextrin broth. *Note* the yeastlike budding cells.

Fig. 4.—A four-weeks-old growth in dextrin broth. *Note* the extensive mycelial development.

B. *Agglutination Absorption Test:* To 5 c.c. of a 1:10 dilution of serum, 5 c.c. of a very heavy suspension of the organism were added. This mixture was shaken for one-half hour and incubated at 37° C. for two hours with occasional shaking. It was then placed in the ice box overnight, centrifuged and the absorbed serum drawn off. Each serum was tested with its homologous organism for completeness of absorption. If this was incomplete, the absorption was repeated. If the absorption had been complete, 0.5 c.c. of the absorbed serum was added to each tube containing 0.5 c.c. of the organism to be tested. It is important especially with monilia to have saline controls of each of the organisms as well as normal rabbit serum controls, because of the tendency to spontaneous agglutination. The results of the agglutination absorption tests of the 22 strains with each of the nine absorbed serums were negative, that is no agglutination took place.

DISCUSSION

A study of Table I reveals that the monilias used in this study are from four main sources: (1) Sputum in pulmonary diseases. (2) Autopsy materials No. 1A, 1B, 1C, 1D, and 2A. (3) Normal throats. (4) Minor upper respiratory conditions.

Eleven of these organisms are definitely known to have their origin from sputums in cases of pulmonary disease; two others, No. 19 and 21, are probably also from pulmonary diseases, as indicated by the information obtained with the cultures. Six of the 11 definitely known organisms isolated from pulmonary diseases (No. 7, 8, 9, 14, 15 and 22) were obtained in the examination of 178 consecutive cases of pulmonary infections. This result indicates that 2.93 per

TABLE IV

1935 BIOCHEMIC REACTIONS AND CARBOHYDRATE UTILIZATION

SOURCE	STRAIN NUMBER	DEXTROSE	LEVULOSE	MALTOSE	D-GALACTOSE	SUCROSE	XYLOSE	INULIN	LACTOSE	DEXTRIN	L-ARABINOSE
Sputum	1	⊕	⊕	⊕	+	+	+	–	–	+	–
Rabbit 1	1A	⊕	⊕	⊕	+	+	+	–	–	–	–
Brain	1B	⊕	⊕	⊕	+	+	+	–	–	–	–
Left lung	1C	⊕	⊕	⊕	+	+	+	–	–	–	–
Right lung	1D	⊕	⊕	⊕	+	+	+	–	–	–	–
Sputum	2	⊕	⊕	⊕	+	+	+	–	–	–	–
Rabbit 2	2A	⊕	⊕	⊕	+	+	+	–	–	–	–
Sputum	3	⊕	⊕	⊕	+	+*	+	–	–	–	±
Sputum	4	⊕	⊕	+	+	+	+	–	–	–	–
Sputum	5	⊕	+	+	+	+	+	–	–	–	+
Sputum	7	⊕	+	+	+	+	+	–	–	–	–
Sputum	8	⊕	+	+	+	+	+	–	+	+	–
Sputum	9	⊕	⊕	+	+	+	+	–	–	–	–
Normal throat	10	⊕	⊕	+	+	+	+	–	–	–	–
Normal throat	11	⊕	⊕	+	+	+	+	–	–	–	–
Normal throat	12	⊕	⊕	+	+	+*	+	–	–	–	+

⊕ Indicates acid and gas, and carbohydrate utilization. – Indicates no acid or gas.
+ Indicates carbohydrate utilization and acid. * Indicates not completely.

cent of all pulmonary diseases may be caused by monilia. In two instances both monilia and *Mycobacterium tuberculosis* were found in the same sputum. The monilias thus found are No. 3 and 6. (No. 6 was lost in 1934.) These two organisms are not included in the six mentioned above as found in 178 sputum examinations. The patients in whose sputums both of these organisms were demonstrated grew progressively worse in spite of the best care and treatment possible. This indicates that a mixed infection of monilia and *Mycobacterium tuberculosis* is a very serious combination.

Three monilia cultures (No. 10, 11, 12) were obtained from the throats of 100 apparently normal individuals, indicating that monilias are found in the throats of 3 per cent of normal persons without causing any apparent ill effects. The organisms recovered from cases originating in miner upper respiratory conditions are No. 13, 16, 17, 18 and 20.

The results of the cultural and morphologic studies of all the monilias recorded in Table I, show practically no difference in either of these characteristics. The only differences observed were in the cases of No. 2, 8, and 13 which produced a very slight alkalinity in litmus milk on one occasion (1937) and a variation in the rate of growth of some of these strains, No. 3 and 18 being rather slow, while No. 4 and 8 were rather rapid in their development.

The ability of the organisms to attack carbohydrates with acid and gas production and acid production alone is demonstrated in Tables II, III, IV, and V. A study of these tables reveals the fact that some of the organisms varied in this respect at the time of their isolation: i.e., No. 1, 1A, 1B, 1C, 1D, 2, 2A, 3, 4, 5, and 6, while No. 7 to 12 inclusive showed variations in subsequent tests. No. 13 to 22, inclusive, were tested only once (1937) and it is there-

TABLE V

1937 BIOCHEMIC REACTIONS AND CARBOHYDRATE UTILIZATION

SOURCE	STRAIN NUMBER	DEXTROSE	LEVULOSE	MALTOSE	D-GALACTOSE	SUCROSE	XYLOSE	INULIN	LACTOSE	DEXTRIN	L-ARABINOSE
Sputum	1	⊕	⊕	⊕	+	+	+	−	−	+	−
Rabbit 1	1A	⊕	⊕	⊕	+	+	+	−	−	−	−
Brain	1B	⊕	⊕	⊕	+	+	+	−	−	+	−
Left lung	1C	⊕	⊕	⊕	+	+	+	−	−	+	−
Right lung	1D	⊕	⊕	⊕	+	+	+	−	−	+	−
Sputum	2	⊕	⊕	⊕	+	+	+	−	−	+	−
Rabbit 2	2A	⊕	⊕	⊕	+	+	+	−	−	+	−
Sputum	3	⊕	⊕	⊕	+	+	+	−	−	−	−
Sputum	4	⊕	⊕	⊕	+	+	+	−	−	+	−
Sputum	5	⊕	⊕	⊕	+	+	+	−	−	+	−
Sputum	7	⊕	⊕	⊕	+	+	+	−	−	+	−
Sputum	8	⊕	⊕	⊕	+	+	⊕	−	−	+	−
Sputum	9	⊕	⊕	⊕	+	+	+	−	−	−	−
Normal throat	10	⊕	⊕	⊕	+	+	+	−	−	−	−
Normal throat	11	⊕	⊕	⊕	+	+	+	−	−	−	−
Normal throat	12	⊕	⊕	+	+	+	⊕	−	−	−	−

⊕ Indicates acid and gas and carbohydrates utilized.
\+ Indicates acid and carbohydrates utilized.
− Indicates no acid or gas and carbohydrates not utilized.

fore unknown whether or not they may vary later in this respect. The most striking variation was demonstrated by the monilia 8 which gained the ability to produce gas from levulose, maltose and sucrose and lost the ability to attack lactose with acid production. Three organisms acquired the power to produce gas from levulose (No. 5, 7, and 8), while eight were able to accomplish this in maltose (No. 4, 5, 7, 8, 9, 10, 11 and 12) and only two in sucrose (No. 8 and 12). Dextrin, arabinose and lactose were not consistently attacked.

A study of Tables IV and V, which present results for the utilization of the seven carbohydrates, dextrose, levulose, maltose, galatose, sucrose, xylose, and inulin, in a method suggested by Lamb and Lamb (1935) reveals the fact that no variation occurred in two tests made in 1935 and 1937 and that all organisms studied utilized all but inulin and thus fall in Group 1 (Lamb and Lamb),

which is comprised of *M. albicans*, *M. psilosis* and *M. candida*. Since these organisms are pathogenic they should be considered as *M. albicans* (*M. pinoyi*, *castellani*).

An examination of Table VI reveals that satisfactory concentrations of both precipitating and agglutinating antibodies were obtained in all of the animals injected in this study, and that the titers of the antiserums for the precipitins were consistently less than those of the agglutinins. This indicates that the specific carbohydrates are not as good antibody stimulators as the proteins. This variation is most strikingly demonstrated by monilia 8. In Rabbit A70 the precipitin titer resulting was 1:50 and in Rabbit A71 it was 1:100, in these same rabbits the agglutinin concentrations obtained were 1:2000 and 1:4000, respectively. The concentration of 1:50 was the lowest obtained for the precipitin serums and 1:4000 was the highest recorded for the agglutinins.

TABLE VI

RABBIT IMMUNIZATION RESULTS

RABBIT NUMBER	STRAIN NUMBER	PRECIPITIN TITER	AGGLUTININ TITER	CONTROL NOR. SERUM	CONTROL SALINE
A58	1	1:100	1: 500	0	0
A59	1	1:250	1: 500	0	0
A60	1A	1:100	1: 500	0	0
A61	1A	1:100	1: 500	0	0
A72	1B	1:100	1: 500	0	0
A73	1B	1:100	1: 500	0	0
A62	1C	1:250	1: 500	0	0
A64	2	1:250	1:1000	0	0
A65	2	1:250	1:1000	0	0
A74	2A	1:250	1: 500	0	0
A75	2A	1:250	1: 500	0	0
A66	5	1:500	1:2000	0	0
A67	5	1:500	1:2000	0	0
A68	10	1:100	1:2000	0	0
A69	10	1:250	1:2000	0	0
A70	8	1:50	1:2000	0	0
A71	8	1:100	1:4000	0	0

Precipitin absorption tests with each of the twenty-two strains with each of the nine absorbed serums developed in this study gave negative results. Not in one instance could any sign of a precipitate be detected. This lack of precipitation indicates that they are all very closely related strains.

Agglutination absorption tests with the 22 strains with the same nine absorbed serums gave the same results as the precipitin absorption tests did. No spontaneous agglutination was observed. It is believed that the use of young cultures (not more than eighteen hours old) and thorough shaking prevents this objectionable reaction.

Agglutinating and precipitating absorption tests with known species of monilias, therefore, furnish a rapid and dependable method of classifying yeastlike fungi (monilia). A slow but also dependable method is that of testing their ability to utilize dextrose, levulose, maltose, galactose, sucrose, xylose, and inulin according to the method of Lamb and Lamb (1935).

SUMMARY AND CONCLUSIONS

The observations made during this study may be summarized as follows:

1. Three per cent of apparently normal individuals in the group tested harbor monilias in their throats.

2. The incidence of pulmonary disease caused by these organisms in 178 cases was 2.93 per cent.

3. The ability to attack carbohydrates with acid or with acid and gas production is not a reliable criterion for the classification of these fungi.

4. Carbohydrate utilization tests according to the method of Lamb and Lamb (1935) showed no variation in two tests, two years apart. The tests showed that all strains studied were identical and that they fall into Group 1 (Lamb and Lamb) and Group 2 (Stoval and Bubolz, 1932). This group is composed of *M. albicans*, *M. psilosis* and *M. candida*.

5. Precipitin and agglutinin absorption tests demonstrated that the organisms were serologically identical.

6. It is believed that the reliable results of the carbohydrate utilization tests combined with precipitation and agglutination reactions furnish a dependable method for the classification of these yeastlike organisms.

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CORONARY DISEASE IN DIABETES MELLITUS*

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IT IS a well-known clinical fact that diabetes mellitus and disease of the coronary vessels are often associated. In 1864 Seegan[1] commented upon the occurrence of angina pectoris and diabetes in one patient. Since then, and especially during the past ten years, there has been accumulated a considerable amount of literature dealing with different aspects of this clinical problem. S. A. Levine[2] believes that among the disease entities that are etiologically related to coronary thrombosis, diabetes is second in importance only to a previously existing hypertension. In 500 cases of diabetic autopsies, I. M. Rabinovitch[3] found that of those individuals whose diabetes lasted five years or more, 80 per cent had cardiovascular disease, regardless of the age of the patient and severity of the disease. Shields Warren[4] states, "I have yet to see a diabetic at autopsy, or to read a protocol of a diabetic, whose disease lasted five years or more, free from arteriosclerosis, regardless of age." Blotner[1] found that of 77 diabetic patients who came to autopsy, 45 per cent had well-marked diseases of the coronary vessels, while of 450 nondiabetics, only 21 per cent had definite lesions in the coronary arteries. Root[5] found advanced coronary artery disease at autopsy twice as frequently in diabetics as in nondiabetics. Of 249 consecutive autopsies above the age of fifty years examined by Nathanson,[6] there were 8.2 per cent showing extensive coronary sclerosis as compared with 52.7 per cent in the diabetic series of 100 autopsies.

The present work represents a study of the histopathologic appearance of the coronary vessels in diabetic and nondiabetic hearts of comparable age periods, and it consists of three parts:

1. Examination and histologic study of coronary vessels in 31 diabetic hearts.
2. Examination and histologic study of coronary vessels in nondiabetic hearts of comparable age periods.
3. Statistical analysis of the incidence of coronary artery disease in diabetic and nondiabetic patients at autopsies at The Mount Sinai Hospital.

*From the Laboratories of The Mount Sinai Hospital.
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Methods and Materials.—The hearts were fixed in 10 per cent formalin-saline according to the technique described by Gross, Epstein and Kugel,[7, 8] and sections were cut from the left coronary artery about 1 cm. from its ostium; one from the left anterior descending branch about 2 cm. from the ostium; one from the right circumflex coronary artery, 1 cm. from its ostium, and one from the posterior descending branch about 1 cm. below the auriculoventricular sulcus. The sections were imbedded in 10 per cent gelatin and kept in the incubator at 38° C. for twenty-four hours, followed by imbedding in 20 per cent gelatin for another twenty-four hours under similar conditions, and then cut by the frozen section method. The cut sections were stained with hematoxylin followed by Sudan III. Duplicate sections were imbedded by the usual paraffin method and stained with hematoxylin-eosin and Weigert's elastica method.

Coronary Arteries of Diabetic Hearts.—The ages ranged between forty-four and seventy-eight, and in addition, there was one patient six years of age and one of twenty years of age. There were 6 cases in the fifth decade, 9 in the sixth decade, 11 in the seventh decade, and 3 in the eighth decade. The six-year-old patient was a boy who was known to have had diabetes for two years and to have died in coma. Macroscopically, the coronary arteries of this heart were smooth and appeared normal throughout; microscopically they showed very mild sclerosis of the intima, but no deposition of fat. The twenty-year-old individual also died in coma and the duration of diabetes was not obtainable. Macroscopically, the vessels of this heart showed a few small yellowish flat plaques, but appeared normal otherwise, while microscopically, they showed only a moderate degree of sclerosis and no fat deposits.

Gross examination of the coronary vessels of the remaining 29 hearts showed mild degrees of sclerotic changes in 8 hearts and marked to extensive sclerosis in the remaining 21 hearts. Of the latter, 7 presented acute thrombotic occlusion of one of the coronary branches. Microscopic sections of the coronary vessels of all of these cases showed well-developed intimal thickening. This intimal thickening varied in width from 3 to 16 times that of the media of the corresponding vessel. The thickening consisted mainly of an increased amount of loose connective tissue, elastic tissue elements, collagen, and the deposition of lipid material, as stained by Sudan III. There were no qualitative differences found either in the different vessels of the same case or in the vessels of the different cases. Neither were there found any significant differences in the vessels obtained from the hearts of different age periods or changes that would be characteristic of the duration or severity of the diabetes. However, there seemed to be a quantitative difference inasmuch as the longer the duration of the diabetes and the older the patient, the more marked were these changes, and the eight cases which grossly presented mild alteration, showed similar changes, but to a lesser degree. These changes were more marked in the left anterior descending branch of the left coronary artery and in the right circumflex coronary artery; they were least developed in the posterior descending branch of the right coronary artery.

Coronary Arteries in Nondiabetic Hearts.—Twenty hearts removed from nondiabetic patients at autopsies, but with well-developed coronary artery disease, were selected for study, in order to compare the lesions found in these vessels with those found in the diabetic hearts of similar age periods. There

were five cases in each of the fifth, sixth, seventh, and eighth decades. The extent and character of the intimal sclerosis and the deposition of lipid material differed in no way from those found in the diabetic hearts. There was not found any single criterion or change that would make it possible to differentiate the coronary vessels of the diabetic heart from those of the nondiabetic heart with well-developed coronary artery disease.

Statistical Analysis of Seven Hundred Consecutive Autopsies.—In Table I, the incidence of coronary artery disease and diabetes mellitus in 700 consecutive autopsies at The Mount Sinai Hospital is classified according to the different decades. It is seen that the highest incidence occurs in the fifth, sixth, seventh, and eighth decades. In the 402 nondiabetic patients between the ages of forty and eighty, 128 hearts with well-developed disease of the coronary arteries were found at autopsy, an incidence of 31 per cent, while among the 29 diabetic pa-

TABLE I

AGE	TOTAL NO. AUTOPSIES	NON-DIABETICS	CORONARY SCL. IN NON-DIABETICS	DIABETES MELLITUS	CORONARY SCL. IN DIABETICS
0-10	108	107		1	
10-20	33	33			
20-30	48	48	3		
30-40	71	70	10	1	
40-50	127	124	20	3	
50-60	126	119	35	7	4
60-70	129	116	59	13	9
70-80	49	43	32	6	5
80-90	9	9	6		
Totals	700	669	165	31	18
40-80	431	402	146	29	18

tients there were 18 hearts with well-developed disease of the coronaries, an incidence of 62 per cent. That is, coronary artery disease was found twice as frequently in the hearts of diabetic as in the hearts of nondiabetic patients.

DISCUSSION

From the foregoing, it is seen that there is no essential difference demonstrable by these methods—Weigert's elastic and sudan fat stain—between vessels of diabetic hearts and those of nondiabetics with well-developed disease of the coronary vessels. It seems likely, therefore, that the pathogenesis of vascular disease found in diabetic hearts is essentially similar to the vascular disease found in the vessels of nondiabetic hearts with well-developed disease of the coronary vessels. This view is supported by the work of Earl R. Lehnherr,[9] who conducted chemical analysis of 25 diabetic aortas, 25 nondiabetic aortas, and 6 children's aortas and found that the changes were similar in the diabetics and nondiabetics, but that there is a greater deposition of lipid, a more marked change in the lipid allocation (increased proportion of the total lipid as cholesterol and a diminished proportion of the total lipid as fatty acid and as phospholipid) and a higher calcium and phosphorus content in the diabetic aorta. The cholesterol content was 8.07 gm. per cent in diabetics as compared with 4.8 gm. per cent in nondiabetics. The source of the lipid remains unknown.

Joslin[10] favors the theory that the cholesterol, which is increased in the diabetic blood, is the basis of the vascular change. Leary[11] claims to have been able to reproduce all the stages of human atherosclerosis in the coronary arteries in the rabbit by feeding cholesterol, and he points out that such lesions do not occur spontaneously in rabbits. The lesions produced varied in different animals in their extent and severity; this variation is comparable to the differences exhibited by human beings. Leary makes the interesting observation that man is the only animal that eats both eggs and milk, the most common sources of cholesterol, throughout his entire lifetime, and man is the only animal that dies in early life from coronary sclerosis. I. M. Rabinovitch[3] also favors the idea that excess blood cholesterol in the circulating blood is an important etiologic factor in the production of arteriosclerosis in the young diabetic. By careful determination of plasma cholesterol, he found an intimate association between cardiovascular disease and hypercholesterolemia, and he states that "deposition of cholesterol and its esters does not necessarily require a high cholesterol content but depends upon the ability of the blood to keep in solution a substance which is probably in a state of supersaturation." In this connection, it is interesting to note the work of F. Rathery and S. Doubrow,[12] who found that the lipoids in the intima are mainly derivatives of palmitic and stearic acid, while in the adventitia, derivatives of oleic acid are abundant.

SUMMARY

1. The microscopic appearance of vascular disease in the coronaries of diabetic hearts is similar to that of nondiabetic hearts with well-developed disease of the coronary vessels.

2. The incidence of coronary artery disease in 29 diabetic patients at The Mount Sinai Hospital upon whom autopsies were performed was 62 per cent as compared with 31 per cent in 402 nondiabetic patients between the ages of forty and eighty.

NOTE: The writer wishes to thank Dr. Louis Gross, Director of the Laboratories at The Mount Sinai Hospital, for his many valuable suggestions.

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THE TREATMENT OF NUTRITIONAL ANEMIA IN RATS WITH MEAT*

LEO K. CAMPBELL, M.D., CHICAGO, ILL.

THREE healthy white female rats, thirty-two days old, were given a diet of only whole milk. The red blood cell count and the hemoglobin as determined by the Sahli method were made at weekly intervals. When the hemoglobin reached its lowest level, meat powder in excess of the daily consumption was placed in the cages with the milk. The meat powder was prepared from ground lean beef round. The meat was then desiccated at 56° C. in a vacuum and pulverized. This method of preparation should not have injured its nutritional value.

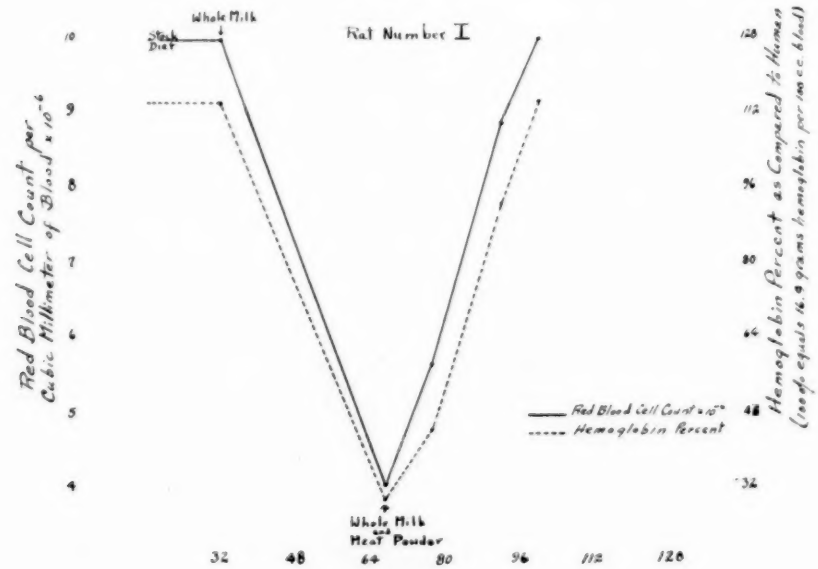

Fig. 1.

In the control group, fifteen rats were fed whole milk and meat powder for one hundred and forty-two days.

In the control group the red blood cell count and hemoglobin remained at normal levels at all times and at the end of the one hundred forty-two days' feeding period the animals were of normal size and in an excellent state of nutrition.

*From the Department of Medicine of Rush Medical College of the University of Chicago and the Presbyterian Hospital in Chicago.

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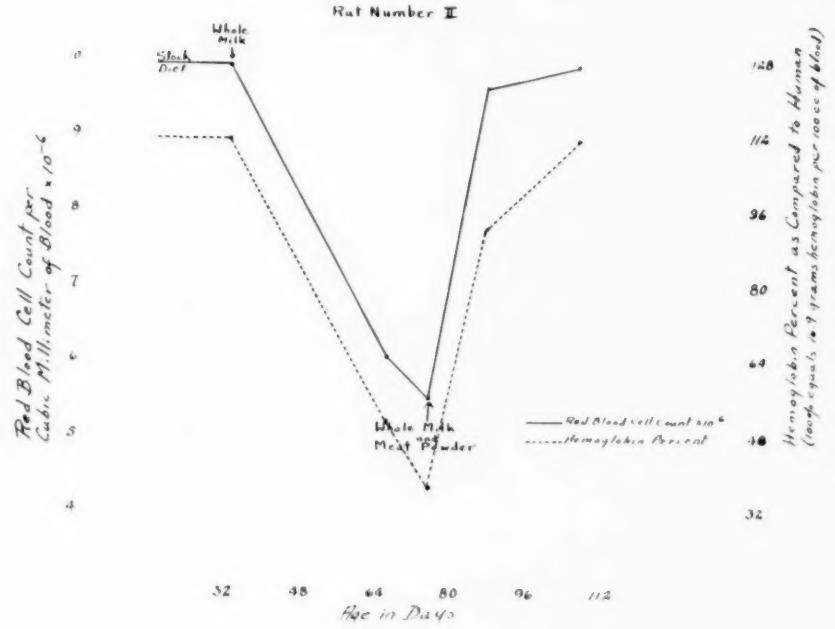
Rat Number II
Whole Milk
Stock Diet
Whole Milk
Meat Powder
Red Blood Cell Count per Cubic M.M. meter of Blood x 10⁻⁶
Hemoglobin Percent as Compared to Human (100% equals 14.9 grams hemoglobin per 100 cc of blood)
Red Blood Cell Count x 10⁻⁶
Hemoglobin Percent
Age in Days

Fig. 2.

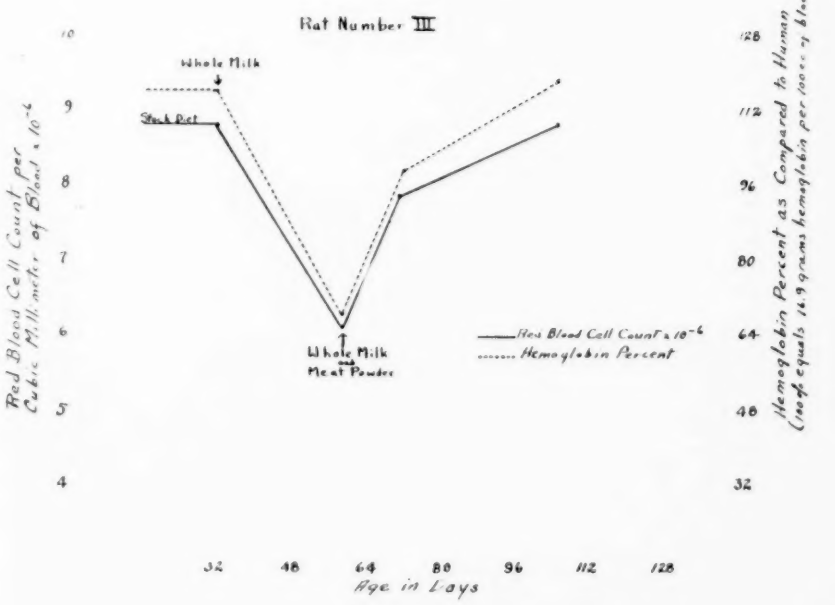
Rat Number III
Whole Milk
Stock Diet
Whole Milk
Meat Powder
Red Blood Cell Count per Cubic M.M. meter of Blood x 10⁻⁶
Hemoglobin Percent as Compared to Human (100% equals 14.9 grams hemoglobin per 100 cc of blood)
Red Blood Cell Count x 10⁻⁶
Hemoglobin Percent
Age in Days

Fig. 3.

RESULTS

RAT	AGE IN DAYS	WEIGHT IN GM.	DIET	DURATION OF DIET IN DAYS	RED BLOOD CELL COUNT PER C. MM. OF BLOOD	HEMOGLOBIN PER CENT AS COMPARED TO HUMAN*	CONDITION OF RED BLOOD CELLS
1	32	52	Stock	Since weaning			
	67	76	Whole milk	35	4,030,000	30	Very hypochromatic, irregular in size and shape
	75	104	Whole milk and meat powder	8	5,510,000	45	Normal
	92	136	Whole milk and meat powder	25	8,920,000	93	Normal
	101	160	Whole milk and meat powder	34	10,000,000	115	Normal
2	32	48	Stock	Since weaning			
	66	84	Whole milk	34	6,080,000	50	Hypochromatic, irregular in size and shape
	75	92	Whole milk	43	5,540,000	38	Very hypochromatic, irregular in size and shape
	87	114	Whole milk and meat powder	12	9,690,000	93	Normal
	107	160	Whole milk and meat powder	32	9,960,000	112	Normal
3	32	48	Stock	Since weaning			
	59	80	Whole milk	27	6,130,000	69	Hypochromatic, irregular in size and shape
	71	88	Whole milk and meat powder	12	7,920,000	100	Normal
	105	128	Whole milk and meat powder	36	8,900,000	119	Normal

*100 per cent equals 16.9 gm. hemoglobin per 100 c.c. of blood.

CONCLUSION

Lean beef from the round is an excellent source of iron for the treatment of secondary anemia produced by malnutrition. The iron apparently is in a readily available form.

THE EFFECT OF HIGH PROTEIN DIETS ON THE KIDNEYS OF RATS*

LEO K. CAMPBELL, M.D., CHICAGO, ILL.

RECENTLY there has been considerable controversy as to the effect of high protein diets in the human kidney. Most clinicians and especially metabolists who are in active contact with nephritic patients believe that a high protein diet will not injure the normal kidney and that such a diet plays no rôle in the etiology of the various forms of inflammatory and degenerative diseases of the kidney. High protein diets have been fed to carnivorous animals without producing anatomic kidney damage and to herbivorous animals with the production of extensive permanent renal injury. As the metabolism of the rat is similar to that of the human, this animal was chosen for the following feeding experiment.

Fifty healthy white female rats from the Wistar strain, 40 only twenty-eight days old and 10 one hundred and twenty days of age, were fed a nutritionally adequate high protein diet for one hundred and fifty days. Whereas the diet of most American people contains approximately 10 per cent of the total energy value as protein calories, the following diet contains 33 per cent of the total fuel value as protein calories. An excess of food was kept in the cages at all times.

FOOD	AMOUNT IN 100 GM. OF RATION	CARBOHYDRATE	PROTEIN	FAT	CALCIUM	PHOSPHORUS	IRON
Whole wheat	38.0	27.4	5.3	0.8	0.017	0.161	0.002
Oats	35.0	23.4	5.6	2.4	0.024	0.139	0.001
Meat powder	15.0	---	13.5	0.9	----	0.083	0.003
Gelatin	5.7	---	5.2	--	----	----	----
Yeast	3.0	1.2	1.4	0.1	0.001	0.042	0.004
Calcium carbonate	1.3	---	---	--	0.520	----	----
Sodium chloride	1.0	---	---	--	----	----	----
Cod liver oil	1.0	---	---	1.0	----	----	----
Ferric citrate	0.01	---	---	--	----	----	0.003
Total	100.00	52.0	31.0	5.2	0.562	0.425	0.013
Caloric value		208.0	124.0	47.0			
Total caloric value ------379							

After feeding for one hundred and fifty days the animals were in fine nutritional condition. They were then killed by gas, the kidneys removed at once, fixed in Zenker's solution, embedded in paraffin, sectioned serially, and stained with hematoxylin and eosin. The serial sections were examined carefully and no evidence of kidney pathology found.

CONCLUSION

A high protein diet when fed to either young or adult normal rats for one hundred and fifty days will not produce anatomical lesions in the kidney.

*From the Department of Medicine of Rush Medical College of the University of Chicago and the Presbyterian Hospital in Chicago.

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THE UTILIZATION OF CALCIUM BY RATS*

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DURING recent years the statement that calcium in forms other than that found in milk is not utilizable by the mammalian organism has gained widespread publicity. Of course clinicians who daily treat parathyroid disease, tetany, Paget's disease, osteomalacia, ulcerative colitis, scurvy, rickets, and allergic diseases with inorganic calcium know the fallacy of the statement. Two years ago during a study in our laboratory of the dietary influence on the kidney function in dogs, six animals were fed only raw meat, cod liver oil, sodium chloride, and calcium carbonate for six months. Two females went through full-term pregnancies. At the end of the experiment the long bones were x-rayed and no evidence of calcium absorption could be found. With this information it was thought advisable to feed two groups of rats, one a diet in which the source of calcium was from a supply other than milk and the other a diet in which milk was a large source of calcium. The calcium contents of the two diets were identical. Tables I and II give the diets used.

TABLE I

DIET A

FOOD	AMOUNT IN 100 GM. OF RATION	CARBOHYDRATE	PROTEIN	FAT	CALCIUM	PHOSPHORUS	IRON
Whole wheat	38.00	27.4	5.3	0.8	0.017	0.161	0.002
Oats	35.00	23.4	5.6	2.4	0.024	0.139	0.001
Meat powder	15.00	---	13.5	0.9	----	0.083	0.003
Gelatin	5.70	---	5.2	--	----	----	----
Yeast	3.00	1.2	1.4	0.1	0.001	0.042	0.004
Calcium carbonate	1.30	---	---	--	0.520	----	----
Sodium chloride	1.00	---	---	--	----	----	----
Cod liver oil	1.00	---	---	1.0	----	----	----
Ferric citrate	0.01	---	---	--	----	----	0.003
Total	100.00	52.0	31.0	5.2	0.562	0.425	0.013
Caloric value		208.0	124.0	47.0			
Total caloric value-----379							

TABLE II

DIET B

FOOD	AMOUNT IN 100 GM. OF RATION	CARBOHYDRATE	PROTEIN	FAT	CALCIUM	PHOSPHORUS	IRON
Whole wheat	30.00	21.6	4.2	0.6	0.014	0.127	0.002
Oats	25.00	16.8	4.0	1.8	0.017	0.099	0.001
Meat	10.00	---	9.0	0.1	----	0.055	0.002
Milk	25.00	9.3	6.6	7.1	0.525	0.250	----
Gelatin	5.00	---	4.6	--	----	----	----
Yeast	3.00	1.2	1.4	0.1	0.001	0.042	0.004
Sodium chloride	1.00	---	--	--	----	----	----
Cod liver oil	1.00	---	--	1.0	----	----	----
Ferric citrate	0.01	---	--	--	----	----	0.003
Total	100.00	48.9	29.8	10.7	0.557	0.573	0.012
Caloric value		195.6	119.2	96.3			
Total caloric value -----411							

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Ten disease-free female rats from the Wistar strain were placed on each diet. The rats were twenty-eight days old and were fed for one hundred and fifty days. An excess of food was kept in the cages at all times.

After blood counts were made, the animals were incinerated and the ash analyzed for calcium by the volumetric oxalate method and for phosphorus by the aminonaphthol sulphonic acid method.

RESULTS

At the end of the experiment the blood count and hemoglobin were normal and all the animals appeared healthy.

TABLE III

CALCIUM AVAILABLE IN SOURCES OTHER THAN MILK

RAT	WEIGHT AT END OF EXPERIMENT IN GM.	WEIGHT OF ASH IN GM.	WEIGHT OF CALCIUM IN GM.	PER CENT OF CALCIUM IN ASH	WEIGHT OF PHOSPHORUS IN GM.	PER CENT OF PHOSPHORUS IN ASH
A	162	6.271	2.509	40	0.413	7
B	180	7.031	2.883	41	0.400	6
C	196	7.241	2.969	41	0.428	6
D	236	8.969	3.767	42	0.400	4
E	200	7.441	3.125	42	0.434	6
F	228	8.260	3.304	40	0.444	5
G	180	7.301	2.701	37	0.405	6
H	176	7.331	3.152	43	0.428	6
I	Died before experiment was completed					
J	Died before experiment was completed					
Average				40		6

TABLE IV

CALCIUM AVAILABLE IN SOURCES INCLUDING MILK

RAT	WEIGHT AT END OF EXPERIMENT	WEIGHT OF ASH IN GM.	WEIGHT OF CALCIUM IN GM.	PER CENT OF CALCIUM IN ASH	WEIGHT OF PHOSPHORUS IN GM.	PER CENT OF PHOSPHORUS IN ASH
AA	196	7.122	2.778	39	0.363	5
BB	190	7.938	3.175	40	0.387	5
CC	196	7.144	2.786	39	0.444	6
DD	184	6.321	2.339	37	0.428	7
EE	188	6.580	2.698	41	0.438	7
FF	204	7.651	3.137	41	0.428	6
GG	206	6.820	2.524	39	0.414	5
HH	188	7.281	3.058	42	0.334	5
II	200	7.230	2.892	40	0.387	5
JJ	168	6.401	2.560	40	0.387	6
Average				40		6

SUMMARY

The calcium and phosphorus content of the ash of rats fed for 150 days on a diet which contained no milk was identical to that of rats which were fed the same diet in which milk was substituted for a part of the food and all of the calcium carbonate.

CONCLUSIONS

From the above data one must conclude that calcium in physical and chemical forms other than that found in milk is utilizable by the mammalian organism.

NORMAL GLUCOSE TOLERANCE TESTS*

BERNARD A. WATSON, M.D., MINNEAPOLIS, MINN.

DURING the last five years at the University of Minnesota Students' Health Service any case of glycosuria occurring during an examination was studied further by a glucose tolerance test.† As this investigation progressed, the question was continually raised as to what constituted a normal glucose tolerance test in healthy young adults. In most studies purporting to give "normal" blood sugar values, the figures have been obtained on a large number of patients both bed and ambulatory in type, of different age, weight, and degree of illness. The mean findings have been determined and assumed to be normal. It seemed important then that a series of glucose tolerance tests be done, large enough to be statistically significant on normal people of a given age group under certain standard conditions. The purpose of this communication is to report such a study. In the literature Gray,[1] whose figures are widely accepted, reported that normally the fasting blood sugar value was 80 to 120 mg. per 100 c.c.; that it should not rise above 180 mg. after ingestion of glucose (the average rise, 140 mg.) and that the blood sugar in three hours should be equal to or below that of the fasting period. In the May 15, 1937, issue of the *Journal of the American Medical Association*[2] in answer to a question on the significance of glycosuria and certain blood sugar values, the following excerpt from the answer is quoted. "A fasting blood sugar of over 120 mg. per hundred c.c. with glycosuria indicates diabetes. The maximum normal blood sugar in a sugar tolerance test (ingestion by an adult of 100 gm. of dextrose) should not exceed 180 mg. at one hour and should drop to 140 mg. at two hours."

Herrman,[3] after a thorough review of the literature, concluded that by using any blood sugar method the fasting blood sugar should not exceed 110 mg.; that the rise after ingestion of glucose should not be more than 200 mg.; and that the blood sugar in two hours should be 110 mg. or less.

TABLE I

(In all tables figures expressed as milligrams of sugar per 100 c.c. of blood)

	MEAN FASTING	½ HOUR	1 HOUR	2 HOURS	3 HOURS
Cutaneous blood sugars	95.0	165	137	97.7	79.9
Venous blood sugars	92.2	151	122	85.4	78.4
Difference between cutaneous and venous sugars	2.8	14	15	12.3	1.5

Cavette and Seljeskog[4] in 1933 reported a comparison of venous and cutaneous sugars obtained during glucose tolerance tests on 21 normal individuals. They had their subjects fast twelve to fourteen hours, after which they obtained a fasting blood sugar and the patient ingested 50 gm. of glucose dissolved in

†A complete analysis of these data will be reported at a later date.

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250 c.c. of water; they then obtained blood sugars at intervals of one-half hour, one hour, two hours, and three hours after the drink. Table I summarizes their results.

Cavette and Seljeskog,[4] as far as can be determined, had the only report in the literature of glucose tolerance tests done on healthy young normal individuals in which the Gibson[5] micromethod was used. This same method was used in our investigation. That certain factors may influence the blood sugar value obtained during a glucose tolerance test is well known. Malmros[6] and Sweeny[7] have shown the effect of diet on the glucose tolerance test. Joslin,[8] Williams and Dick,[9] Von Nordeen and Isaacs[10] have demonstrated that certain diseases, infections or metabolic disturbances may cause a rise in the glucose tolerance curve. Malmros[6] has pointed out the effect of age and Staub[11] has drawn attention to the influence of moderate exercise. Our experience in common with that of other observers is that some drugs taken preceding the test may raise blood sugar values. Because the aforementioned factors may affect the results obtained in glucose tolerance tests, it is obvious that they should be eliminated before the results obtained can be interpreted as normal. Hence an individual consulting a physician in his office or an out-patient clinic cannot be considered "normal" and should not serve as a subject for the determination of normal blood sugar values during a glucose tolerance test.

Up to 1932 there had been no reports in the literature of blood sugar determinations by the Gibson micromethod during a glucose tolerance test on normal young adults.* This fact prompted the beginning of the investigation, the results of which are reported in this communication.

PROCEDURE

Any student volunteering as an experimental subject was questioned as to age, family history of diabetes, weight, diet, previous history of glycosuria, drug ingestion, and general health. If the subject gave no history of previous glycosuria or drug ingestion, was eating a normal diet, and, on a complete physical examination, was found to be in good health, he or she was then given a glucose tolerance test.

TECHNIQUE

The subject ate dinner at 6:00 P.M. the evening preceding the test, spent a quiet evening and had nothing by mouth until 8:00 A.M. on the day of the test. At that time fasting venous blood and urine samples were obtained. The patient was then given 100 gm. of glucose dissolved in 250 c.c. of water, blood and urine samples were taken one-half hour, one hour, two hours and two and one-half hours after the drink, and analyzed for glucose.† The subject was kept at rest during the test and water intake was restricted to three ounces after each blood sugar.

Two laboratory technicians were employed in this investigation, one doing blood sugar analyses on 38 subjects, and the other analyzing results on 45.

*Normal young adult in this instance means that there was no history of glycosuria, presence of disease, or a disproportionate amount of carbohydrate, fat or protein in the diet.

†Gibson micromethod for blood sugar. Benedict's qualitative test for urinary sugar.

DATA

Of the total number of normal subjects given glucose tolerance tests (83), 77 were males and 6 were females (Table II). The ages of this group ranged from eighteen to twenty-eight, the average age was 20.45 years. A family history of diabetes was present in 10 subjects. Glycosuria during the glucose tolerance test occurred in nine subjects, only two of whom had a family history of diabetes. There were 49 males in the normal weight group, 20 in the overweight group, and 8 in the underweight group. The average daily diet consisted of: Carbohydrate, 300 to 400 gm.; fat, 90 to 120 gm.; and protein, 60 to 90 gm.

TABLE II

RESULTS OF BLOOD SUGAR DETERMINATIONS ON 83 NORMAL CASES

	FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Mean	92.00	134.87	114.64	91.51	83.55
Standard deviation	12.45	24.71	29.30	19.72	18.06

TABLE III

GLUCOSE TOLERANCE TESTS OF 10 NORMAL PERSONS WITH A FAMILY HISTORY OF DIABETES

	FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Mean	93.3	143.7	125.6	90.1	74.4

TABLE IV

MEAN BLOOD SUGAR VALUES OF THE NINE INDIVIDUALS WHO SHOWED GLYCOSURIA ON ONE OR MORE OCCASIONS DURING THE GLUCOSE TOLERANCE TEST

	FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Mean	96.88	155.66	155.0	106.44	88.55

DISCUSSION

To determine normal blood sugar values for the glucose tolerance test the first requisite is a normal subject, e.g., one free from disease and on a known diet. A second prerequisite is an accurate blood sugar method and competent technicians. The close approximations of the results obtained by the two technicians doing this study and the consistent results one may obtain by the Gibson method are demonstrated in Table V. The mean blood sugar values obtained in this investigation are below those generally accepted as normal in the literature.

TABLE V

COMPARISON OF THE RESULTS OF TWO TECHNICIANS

		FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Technician A	Mean	90.64	135.94	111.94	90.50	82.50
38 cases	Standard deviation	8.91	20.15	23.39	18.20	17.18
Technician B	Mean	92.71	132.76	115.51	90.78	83.29
45 cases	Standard deviation	14.99	26.25	29.17	18.23	12.03

This study was started in the hope that the findings would enable one to predict whether or not any single glucose tolerance test was abnormal or varying within normal limits. When the mean values and variation from the means

had been determined statistically for the normal group their tolerance tests as well as all initial tolerance tests being done routinely on subjects with glycosuria (this study mentioned in opening paragraph) were placed in one of the five groups listed below.

Group I in which the highest blood sugar after ingestion of glucose was from two to three or more standard deviations from the calculated normal mean (Table II).

Group II in which at least two of the blood sugars during the test were two to three or more standard deviations above the corresponding mean of the normal series.

Group III in which at least three of the blood sugar values were two to three or more standard deviations above the corresponding mean of the normal series.

Group IV in which at least four of the blood sugar values were two to three or more standard deviations above the corresponding mean of the normal series.

Group V in which all five blood sugars were two to three or more standard deviations above the normal mean of the series.

The normal group and some hundred and fifty initial glucose tolerance tests were so classified and were studied further by means of other glucose tolerance tests at intervals of six to twelve months over a period of two to four years. One could assume that a subject with a tolerance test classed in Group I would have less chance of developing a further disturbance of tolerance than an individual with a Group III curve and conversely an individual with a Group V test would have a much less chance of recovering a normal test than one in Group II or III, etc. This assumption in the main was found to be correct but not in a large enough number of cases so that the group classification could be used accurately to determine whether or not any given test was normal and that the individual with such a test need pay no further attention to it.

Numerous statistical formulas were then applied to the initial curve to determine whether or not they could accurately predict the then known outcome of any given case. Dr. Treloar,[12] Assistant Professor of Biometry at the University of Minnesota, was one who suggested certain formulas that might be used. It should be noted that the behavior of each succeeding tolerance test had already been determined during the years this study was being carried out so that there was a definite check on the efficacy of each formula tried. It was found the more complex the formula the less liable it was to predict the correct outcome. From this statistical study, however, I discovered one formula which accurately predicted whether or not one was dealing with a normal tolerance test in about ninety to ninety-five per cent of the cases studied. The formula is as follows: x = fasting + highest + 2½ hour blood sugar values expressed in mg. per hundred c.c. of blood divided by three. In interpreting the results if x is above 140 the glucose tolerance test should be classed as abnormal until proved otherwise by further tests and clinical observations. In border line cases just above or below 140 one should study further any individual case before a final conclusion is drawn. If the figure is above 145 to 150, one is almost certain to be dealing with a disturbance in glucose tolerance. Since the original observations on one hundred and fifty tolerance tests were done the formula has continued to aid the writer in his interpretation of over five hundred tests, thus its depend-

ability seems established. It should be remembered, however, that any formula may fail in any single instance.

Other incidental though interesting findings in this study are the high incidence 10.8 per cent (9 of 83) of glycosuria occurring during a tolerance test on healthy young adults (Table IV). The fact that normal people with family history of diabetes do not have evidence of a disturbed glucose tolerance test has been frequently observed.

TABLE VI

RESULTS OF GLUCOSE TOLERANCE TESTS ON MALE SUBJECTS, MALE SUBJECTS IN NORMAL, OVER- AND UNDERWEIGHT GROUPS*

		FASTING	½ HOUR	1 HOUR	2 HOURS	2½ HOURS
Normal weight 49 cases	Mean	91.17	135.61	114.38	88.67	82.96
	Standard deviation	12.27	25.90	27.51	19.24	19.89
Overweight 20 cases	Mean	94.50	135.50	122.00	95.50	83.50
	Standard deviation	9.90	25.19	34.65	19.61	12.20
Underweight 8 cases	Mean	91.50	122.75	98.63	93.50	81.63
	Standard deviation	17.99	15.70	23.15	16.10	17.68

*Normal weight=90 to 110 per cent of normal weight as calculated from standard insurance tables.

Overweight=Over 110 per cent of normal weight as calculated from standard insurance tables.

Underweight=under 90 per cent of normal weight as calculated from standard insurance tables.

Comparing the normal, over- and underweight groups (Table VI) one sees that in the first two groups the half-hour blood sugar values are the same while in the underweight group it is lower. No conclusions can be drawn from these findings as the series is too small. It is interesting, however, that the overweight group's blood sugar values are practically the same as the normal weight group's.

CONCLUSIONS

1. Eighty-three subjects between the ages of eighteen and twenty-eight, in good health and on a diet of 300 to 400 gm. of carbohydrates, 90 to 120 gm. of fat, and 60 to 90 gm. of proteins, were given a glucose tolerance test. The mean blood sugar values (expressed in mg. per 100 c.c. blood) were: Fasting, 92; ½ hour, 134.8; 1 hour, 114.64; 2 hours, 91.51; 2½ hours, 83.55.

2. The figures are below those generally accepted as normal.

3. Statistical formulas cannot be applied to a single glucose tolerance test to predict accurately the ultimate clinical prognosis of a patient with a disturbed carbohydrate metabolism. The formula:

$$x = \frac{\text{fasting} + \text{highest} + 2\frac{1}{2}\text{ hr. blood sugar values expressed in mg./100 c.c. blood}}{3}$$

is more accurate than any other statistical formula that was tried. If x exceeds 140, the patient should be observed for evidence of further disturbance of the glucose tolerance test.

4. The Gibson micromethod of blood sugar determination is a satisfactory method.

5. One finds on doing glucose determinations on urines collected during the glucose tolerance test that glycosuria is not uncommon, e.g., 9 cases or approximately 11 per cent of the group showed glycosuria during the test.

6. Although the overweight group is small (20 cases), it seems that in young adults obesity does not predispose the individual to a disturbed carbohydrate metabolism.

7. In our series subjects with family history of diabetes showed no evidence of diminished carbohydrate metabolism as determined by the glucose tolerance test.

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HYPERPROTEINEMIA IN MULTIPLE MYELOMA*

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HYPERPROTEINEMIA as a manifestation of multiple myeloma has been reported so frequently in the past few years that it is being considered as one of the important points in the differential diagnosis of this condition. This association was first emphasized by Perlzweig, Delrue and Geschickter[1] in 1928 although Jacobson[2] in 1917 found 7.86 per cent of a substance he believed to be Bence-Jones protein in blood obtained from the heart of a patient who had died of multiple myeloma and Pribram[3] in 1925, reported a case with a total protein of 8.7 per cent. We have been able to find 52 cases of multiple myeloma with hyperproteinemia almost all of which have been reported since 1928 (Table I).

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The normal values for plasma proteins range from 6 to 8.7 per cent,[7, 15 35-37] so that a total plasma protein of 9 per cent or over may be regarded as a definite increase. We have included in Table I only those cases in which the protein content of the plasma reached or exceeded this figure. The highest total protein reported was 18.37 per cent (Case 32). In those cases in which a protein partition was done the albumin fraction was usually found to be within normal limits but the globulin fraction was consistently increased. The fibrinogen varied from normal to very high levels. Complete absence of serum albumin was found in one case (Case 38), but the patient's blood fibrinogen was 8.42 per cent. Although extremely high globulin values were occasionally found, there is no agreement as to which globulin fraction is responsible for this increase. It has been shown that the accurate differentiation of euglobulin and pseudoglobulin I and II is difficult[38] and that the results may vary with a difference in the dilution of the serum[39] and with different techniques.[39] In the reported cases the euglobulin was usually greater than the pseudoglobulin, although the reverse was true in Cases 36 and 39. The hyperproteinemia apparently is not due to the presence of Bence-Jones protein, for most investigators have not been able to detect its presence. However, Jacobson[2] found 7.86 per cent of Bence-Jones protein in blood removed postmortem and Cantarow[26] found 5.2 per cent in a case (Case 40) with a total protein of 11.3 per cent. Shirer, Duncan, and Haden[8] apparently demonstrated Bence-Jones protein in large amounts in their two cases. Gabbe[40] detected 0.42 per cent of this protein in the blood of a patient who showed 30 per cent Bence-Jones protein in the urine and small amounts have been found in the blood by other investigators.[41-45] It has been suggested that Bence-Jones protein is easily excreted by the kidney due to its relatively small molecular weight and therefore does not accumulate in the blood.[29] Bence-Jones proteinuria was sought for in 35 of the reported cases and found in 23 of them.

The general incidence of hyperproteinemia in multiple myeloma cannot be ascertained accurately from Table I for these patients were deliberately selected for report because they showed hyperproteinemia. The blood protein may reach or exceed 9 per cent not only in multiple myeloma, but also in kala-azar,[46] syphilis,[46] myelogenous leucemia,[25] "a toxic infectious lesion of the central nervous system,"[47] lymphogranuloma inguinale,[34] large kidney tumors,[48] sarcoid of Boeck,[49] and in lymphosarcoma.[34] In Rowe's case of inguinal lymphadenopathy[50] the total protein was found to be approximately normal but rose to 10.4 per cent after venous stasis had been produced in the arm.

The blood protein studies which we made on 10 patients with multiple myeloma are summarized in Table II. It will be noted that hyperproteinemia (total protein over 9 per cent) occurred in 3, or 30 per cent of the 10 cases. In 3 additional cases (Cases 5, 6, and 7) the total protein was over 8 per cent, but these values must be considered within the upper limits of normal. In the cases with a distinct hyperproteinemia, the albumin fraction was either normal or slightly below and the increase in the total protein was due chiefly to globulin. The fibrinogen was increased in Cases 2, 7, and 8 but was approximately normal in the others. In all but the first 3 cases there was an alteration in the normal albumin-globulin ratio, with actual inversion of the ratio in four instances (Cases

TABLE I

BLOOD PROTEIN STUDIES FROM THE LITERATURE

AUTHOR	YEAR	PLASMA OR SERUM	TOTAL PROTEIN GM. PER CENT	ALBUMIN GM. PER CENT	GLOBULIN GM. PER CENT	EUGLOBULIN GM. PER CENT	PSEUDO-GLOBULIN GM. PER CENT	FIBRINOGEN I AND II GM. PER CENT	BENCE-JONES PROTEIN IN URINE
1. Jacobson[2]	1917		7.86*		–	–	–	–	+
2. Perlzweig[1]	1928		13.84	4.06	9.09	6.79	0.63	1.49	0.69	+
3. Bannick[4]	1929	Serum	9.00	2.50	6.50	–	–	–	–	+
4. Bannick	1929	Serum	10.54	4.45	6.09	4.40	1.14	0.55	–	+
5. Johansen[5]	1931	Serum	11.00	0.36	0.48	5.83	0.97	1.69	0.92	0
6. Jores[6]	1931	Blood	9.32	3.32	5.81	–	–	–	0.19	+
7. Kumpf[7]	1931	Plasma	14.13	4.65	9.48	–	–	–	1.45	0
8. Shirer[8]	1932	Serum	13.78	2.44	11.34	–	–	–	–	0
9. Shirer	1932	Serum	10.02	2.30	7.72	6.32	0.66	0.74	–	+
10. Reimann[9]	1932	Plasma	10.12	0.90	9.10	2.37	1.47†		5.48	0
11. Medes[10]	1932	–	16.00	–	–	–	–	–	–	–
12. Bennhold[11]	1932	Serum	11.40	1.80	9.60	–	–	–	–	–
13. Bennhold	1932	Serum	9.50	2.60	6.90	–	–	–	–	–
14. Bennhold	1932	Serum	16.00	1.20	14.80	–	–	–	–	–
15. Veil[12]	1932	Serum	14.00	6.20	7.90	–	–	–	–	–
16. Magnus-Levy[13]	1933	Serum	9.90	3.30	6.60	4.10	2.50†		0.50	+
17. Magnus-Levy	1933	Serum	13.00	3.50	9.50	7.30	2.20†		0.50	+
18. Magnus-Levy	1933	Serum	12.00	4.70	7.30	6.10	1.20†		–	+
19. Veil[14]	1933	Serum	12.10	5.70	7.0	–	–	–	–	+
20. Peters[15]	1933	Serum	High	–	–	–	–	–	–	–
21. Peters	1933	Serum	High	–	–	–	–	–	–	–
22. Bönniger[16]	1933	Blood	13.00	–	–	–	–	–	–	–
23. Wintrobe[17]	1933	Plasma	11.90	–	Increased		–	–	–	0
24. Herbert[18]	1933	–	12.6	–	–	–	–	–	–	–
25. Johansen[19]	1934	Plasma	10.80	2.08	8.72	–	–	–	–	0
26. Johansen	1934	Plasma	11.00	0.36	8.48	5.83	.96	1.68	0.92	0
27. Johansen	1934	Plasma	13.50	1.96	10.15	8.93	.53	.68	1.32	+?
28. Johansen	1934	Plasma	11.39	1.96	9.14	7.25	1.45	.34	0.28	0
29. Foord[20]	1935	Plasma	13.20	4.30	8.30	–	–	–	0.60	0
30. Foord	1935	Plasma	15.00	5.50	8.80	–	–	–	–	+
31. Foord	1935	Plasma	11.37	1.86	9.51	–	–	–	–	–
32. Foord[21]	1935	Plasma	18.37	6.00	11.90	–	–	–	0.47	+
33. Foord	1935	Plasma	12.74	2.87	9.38	–	–	–	0.49	+
34. Sweigert[22]	1935	Plasma	12.00	1.80	10.20	–	–	–	0.75	+
35. Bauer[23]	1935	Plasma	11.66	2.94	8.72	4.41	4.31†		0.31	0
36. Jacobson[24]	1935	Serum	10.50	1.60	8.90	3.10	5.80†		–	–
37. Jacobson	1935	Serum	11.10	2.20	8.90	7.90	1.00†		–	–
38. Jacobson	1935	Serum	12.70	0.00	12.70	10.00	2.70†		–	–
39. Gros[25]	1935	Serum	11.68	2.54	9.14	.12	5.34	3.68	0.28	0
40. Cantarow[26]	1935	Serum	11.30	2.80	8.50	–	–	–	–	+
41. Cabot Case[27]	1935	Serum	11.70	–	–	–	–	–	–	+
42. Robbins[28]	1935	Serum	13.10	2.80	10.30	–	–	–	–	0
43. Berglund[29]	1935	Plasma	16.10	2.50	–	7.41	0.59	0.38	5.22	+
44. Berglund	1935	Plasma	17.58	1.16	–	4.58	2.50	0.51	8.42	+
45. Geschickter[30]	1936	Plasma	11.07	–	–	–	–	–	–	–
46. Cabot Case[31]	1936	Serum	11.40	–	–	–	–	–	–	+
47. Gutman[32]	1936	Serum	9.00	–	–	–	–	–	–	+
48. Gutman	1936	Serum	9.90	–	–	–	–	–	–	+?
49. Bing[33]	1936	Serum	11.02	2.86	8.16	–	–	–	–	0
50. Bing	1936	Serum	12.69	3.07	9.62	–	–	–	–	+
51. Gutman[34]	1936	Serum	10.09	3.5	7.4	4.6	–	–	–	–
52. Gutman	1936	Serum	9.9	2.9	7.0	–	–	–	–	–

*Reported as Bence-Jones protein: total protein not determined.
†Total for I and II.
– Information not available.

TABLE II

CHEMICAL STUDIES IN 10 CASES OF MULTIPLE MYELOMA

	AGE	SEX	STAGE OF DISEASE WHEN THE STUDIES WERE MADE	SERUM OR PLASMA	TOTAL PROTEIN GM. %	ALBUMIN GM. %	GLOBULIN GM. %	FIBRINOGEN GM. %	BENCE-JONES BODIES IN URINE	SERUM CALCIUM MG. %	SERUM PHOSPHORUS MG. %	RENAL FUNCTION	CELL TYPE
1	48	M	4 mo. after onset of symptoms	P	6.85	3.65	2.76	0.44	0	10.20	3.20	Blood nitrogen normal	–
2	49	M	1st year of a 6-year course	P	7.38	4.61	1.85	0.92	+	12.80	4.10	–	–
3	45	F	3rd year of a 4-year course	S P P	7.00 7.17 6.37	5.40 3.38 3.59	1.60 3.32 2.18	– 0.47 0.60	0	10.70	3.40	Blood nitrogen normal Urea clearance 116% of normal Maximum specific gravity of urine 1.024	Biopsy of scapula, plasma cell type
4	51	M	1 year after onset; 1 mo. before death	P	7.53	3.71	3.21	0.61	+	15.00	5.00	–	–
5	57	M	1 mo. before death; 1 year from onset	P P P	6.85 8.05 8.00	3.21 3.96 2.60	3.77 3.54 4.97	0.56 0.55 0.43	+	9.75	2.86	Blood urea nitrogen 25.9 mg. % Urea clearance 58% of normal Maximum specific gravity of urine 1.034	Biopsy of bone, plasma cell type

TABLE II—CONT'D

6	57	M	1 mo. before death; 3 years from onset	P	8.37	3.53	4.16	0.68	+	13.40	2.86	Blood nitrogen normal Urea clearance 46% of normal Maximum specific gravity of urine 1.011	Autopsy, plasma cell type
7	28	F	2 days before death; 6(?) years from onset	P P P	8.30 8.00 8.86	4.70 4.80 3.76	3.60 2.20 5.10	1.20 1.00 –	0	15.90	3.74	Blood nitrogen normal Urea clearance 40% of normal Maximum specific gravity of urine 1.017	Autopsy, plasma cell type
8	66	M	Pt. bedridden 2 mo. later; 2 years from onset	P S	9.04 9.15	3.01 2.68	4.58 6.47	1.45 –	?	11.2	4.20	Blood urea nitrogen 21.0 mg. % Blood creatinine 1.8 mg. % Blood uric acid 5.1 mg. %	–
9	52	F	2 years from onset of symptoms	S S P S	12.30 10.30 9.13 12.40	1.50 1.40 2.00 2.10	10.80 8.90 6.61 10.10	 0.52 0.20	+	17.1 16.7	2.66 2.80	Blood nitrogen normal Urea clearance 26% of normal Maximum specific gravity of urine 1.013	Biopsy of skull, blast cell type
10	54	F	10 days before death; 6 mo. from onset	P P	10.00 11.59	3.23 3.05	6.39 8.09	0.38 0.45	0	10.6	4.3	Blood creatinine 10.2 mg. % Blood uric acid 5.4 mg. % Blood urea nitrogen 64.0 mg. %	Autopsy, plasma cell type

Blood protein determinations were done by Howe's method: J. Biol. Chem. **49**: 93 and 109, 1921 and by the colorimetric method of Andersch and Gibson with a corrected factor: J. LAB. & CLIN. MED. **18**: 816, 1933.

6, 8, 9, and 10). The blood was not examined for Bence-Jones protein, and no attempt was made to fractionate the globulin. Examination of the urine revealed Bence-Jones protein in five cases.

Hypoproteinemia has been reported in multiple myeloma by Chester.[51] In two cases he found a total serum protein of 3.58 per cent and 4.74 per cent, respectively. He believed that all cases of multiple myeloma would eventually show these low values if the studies were carried out late in the course of the disease and in support of this he cited the case of Thannhauser and Krauss[52] in which the blood protein fell from 6.12 per cent to 4.49 per cent in four months. Our observations do not substantiate this idea because, although the majority of the determinations were made in an advanced stage of the disease, they gave normal, or high, values. In Case 7, with a proteinemia of 8.86 per cent, the determinations were made two days before death, and in Case 10 the determinations made ten days before death showed 11.59 per cent total protein. In Cases 4, 5,

TABLE III

BLOOD CHEMICAL STUDIES IN VARIOUS CONDITIONS INVOLVING THE SKELETAL SYSTEM

	AGE	SEX	DIAGNOSIS	TOTAL PROTEIN GM. %	ALBUMIN GM. %	GLOBULIN GM. %	FIBRINOGEN GM. %	SERUM CALCIUM MG. %	SERUM PHOSPHORUS MG. %
1	41	M	Carcinoma metastatic from prostate	4.81	2.32	1.98	0.50	12.50	4.00
2	63	M	Carcinoma metastatic from prostate	8.00	3.90	4.10	–	10.80	3.40
3	55	F	Carcinoma metastatic from breast	5.62	2.14	3.36	0.12	10.00	3.70
4	45	F	Carcinoma metastatic from breast	6.05	2.69	2.89	0.50	9.50	4.80
5	58	F	Carcinoma metastatic from breast?	6.06	4.17	1.84	–	10.10	3.50
6	30	M	Carcinoma metastatic from stomach	6.30	–	–	–	18.50	4.60
7	14	M	Ewing's tumor	8.8	–	–	–	12.80	1.94
				7.30	2.80	4.20	0.30		
8	18	M	Ewing's tumor	6.35	3.00	2.78	0.57	19.30	3.60
9	4	M	Myeloblastoma	5.77	2.80	2.68	0.28	9.20	4.40
10	52	F	Tumor, metastatic from brain, type?	7.11	3.68	2.96	0.47	12.20	3.30
11	27	M	Myeloid leucemia	7.30	3.50	3.40	0.40	10.10	3.70
12	43	F	Acromegaly	6.50	2.60	3.90	–	10.80	3.94
13	55	M	Acromegaly	6.90	2.54	3.36	–	10.40	3.90
14	68	F	Senile osteoporosis	7.70	3.10	4.60	–	9.85	3.64
								13.60	–

and 6 the total protein was normal one month before death. Although we cannot be sure that a drop in the blood protein had not occurred, it is evident that hypoproteinemia was not present.

There are several phenomena which may be encountered in the routine examination of patients that suggest the presence of hyperproteinemia. Unusual rouleaux formation or actual autohemagglutination occurring on wet or dry blood smears was noted by Reimann[9] and by Foord.[20] Difficulty or inability to count the erythrocytes because of the formation of a granular precipitate when the blood was mixed with Hayem's solution was observed by Reimann,[9] Bön-

niger,[16] Jacobson,[24] and Foord.[20] The appearance of a heavy milky precipitate when blood serum was heated preparatory to doing a Wassermann test was noted by several investigators.[8, 43, 44] Unusually rapid coagulation of the blood was reported by Johansen.[19] In Case 10 of our series there was marked rouleaux formation on fixed blood smears but no difficulty was encountered in doing either the erythrocyte count or the Wassermann test.

Table III gives the results of blood chemical studies in 14 cases with other types of skeletal lesions. Hyperproteinemia (9 per cent or more) did not occur, although the total protein reached 8.8 per cent in one case of Ewing's tumor. Absolute hyperglobulinemia was not found, but the albumin-globulin ratio was reversed in 7 cases. The fibrinogen values were all within normal limits. The fact that clearly defined hyperproteinemia and hyperglobulinemia seem to be limited to multiple myeloma may be useful in the differential diagnosis of multiple lesions involving the skeletal system.

Serum calcium and phosphorus determinations in the 10 cases of multiple myeloma are shown in Table II. It will be noted that in 5 of them there was an increase of the serum calcium to 12 per cent or over, and that in 3 of these 5 cases there was an associated increase in the inorganic phosphorus. In the other 7 cases the phosphorus values were either normal or slightly above. These observations are in agreement with those of Gutman and others[32] who recently reported 4 cases of multiple myeloma with an increased serum calcium but with normal or slightly elevated phosphorus values. In an extensive review of the literature they collected 36 additional cases presenting similar features. Reference to Table III, however, shows that this is not limited to multiple myeloma, for marked hypercalcemia was present in one case of Ewing's tumor (19.30 per cent) and in a case of carcinoma metastatic from the stomach (18.50 per cent). In neither of these was the serum phosphorus greatly altered. These observations are of value in differentiating multiple myeloma or other malignancies involving the skeletal system from hyperparathyroidism in which there is, as a rule, an increased blood calcium but a lowered phosphorus content.[32]

Renal function studies or blood nitrogen estimations were made in 8 of the 10 cases in our series (Table II). Decreased ability of the kidney to concentrate the urine was present in Cases 6 and 9 and a lowered urea clearance was found in Cases 5, 6, 7, and 9. One patient (Case 10) presented all the manifestations of renal insufficiency and died in uremia with a creatinine of 10.2 and a urea nitrogen of 64 mg. per cent. The blood pressure was normal and there was no clinical evidence of arteriosclerosis. Repeated examination of the urine showed a persistent albuminuria but Bence-Jones protein was never detected. Histologic examination of this patient's kidneys revealed only a minor degree of arterial thickening. A majority of the glomeruli showed no appreciable alteration and only a very few were hyalinized. There was an extensive increase in the interstitial fibrous tissue and a very slight amount of chronic inflammatory reaction. The most striking alterations occurred in the tubules. Many of these contained dense hyaline casts, some of which were surrounded by zones of loose granular hyalin débris, but no material of a crystalline nature was found in the casts. A few giant cells of the foreign body type were located adjacent to the casts and within the lumina of the tubules. Other casts were surrounded by zones of desquamated tubular epithelium and clusters of neutrophiles. Some tubules were

dilated but contained no casts whereas others were constricted and atrophic. Beneath the pelvic epithelium there were lymphocytes, plasma cells and a few eosinophiles. It is possible that some of the kidney scarring was the result of an ascending infection but it seemed probable that the significant changes were associated with the casts in the tubules.

Hamman[53] stated that renal insufficiency with nitrogen retention but without an elevated blood pressure was characteristic of multiple myeloma, and that under these circumstances a careful roentgenologic study of the skeletal system should always be made. Geschickter and Copeland[30] estimate that renal lesions are present in 70 per cent of the cases of multiple myeloma. Although the blood pressure was usually normal, hypertension occurred occasionally. They

TABLE IV

BLOOD STUDIES IN 10 CASES OF MULTIPLE MYELOMA*

	HEMOGLOBIN (SAHLI) PER CENT OF NORMAL	ERYTHROCYTES, MILLIONS	LEUKOCYTES	NEUTROPHILES	EOSINOPHILES	BASOPHILES	LYMPHOCYTES	MONOCYTES	DEGENERATED CELLS	PATHOLOGICAL LYMPHOCYTES	MYELOCYTES	NORMOBLASTS	UNCLASSIFIED CELLS
1	85	3.53	5,200	65	1	1	26		7				
2	87	3.91	3,050	56	2		24	5	1	12			
3	94	4.76	8,800	55			12	3	3	37			
4	35	1.59	11,100	65			22		2		12	2	3
5	47	1.95	3,300	56			41	3					
6	64	2.72	6,900										
7	78	3.54	8,400	79			16	5					
8	87	3.80	7,800	67	3		26	4					
9	39	2.26	4,500	48	1		40	3	4		4		
10	22	1.19	2,850	44			56						

*These cases are in the same order as in Table II.

found also that the kidney was smooth and white and on microscopic examination showed pronounced tubular degeneration. They attributed contraction of the kidney and glomerular changes to a secondary sclerotic process. Bell[54] in 1933 described the kidney changes in eleven cases of multiple myeloma and thoroughly reviewed the literature on this phase of the subject. He found that renal insufficiency was a frequent complication in the advanced stages of multiple myeloma. In his opinion, the only direct effect of multiple myeloma on the kidney is the formation of casts composed of Bence-Jones protein which obstruct the tubules and cause them to become atrophic. Obstruction of large numbers of the tubules leads to extensive atrophy of the cortex and, eventually, to renal insufficiency. The renal changes which we found in our case were the same as those described by Bell[54] in so far as the histological alterations are concerned, but it is doubtful if the tubular casts were composed of Bence-Jones protein, for we were unable to demonstrate the presence of this protein in the urine in spite of repeated attempts to do so.

The hematologic features of multiple myeloma are not striking and are in no way diagnostic (Table IV). The severity of the anemia will depend upon the amount of bone marrow involvement and the duration of the disease. The hemoglobin and erythrocytes are diminished about equally with the result that the color index approaches unity. In our cases the total leucocyte count varied

from 2,850 to 11,100, and there was no discernible relationship between the leucopenia and the degree of reduction in the number of the erythrocytes. Two patients (Cases 4 and 9) showed a few myelocytes in the peripheral blood and in Case 4 nucleated erythrocytes were present. Although a majority of the cases presented an essentially normal differential leucocyte count, it must be realized that abnormal cells appear occasionally.

SUMMARY AND CONCLUSIONS

1. Hyperproteinemia was found in 3 of our 10 cases of multiple myeloma and in addition we have collected 52 cases from the literature.

2. The fact that hyperproteinemia and hyperglobulinemia are practically limited to multiple myeloma may be useful in the differential diagnosis of multiple lesions involving the skeletal system.

3. Hypercalcemia together with a normal or slightly increased inorganic serum phosphorus is frequently found in multiple myeloma, but may occur with other bone lesions.

We are indebted to Doctor R. E. Shaw for the pathologic observations upon the kidney.

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AN UNUSUAL BACILLUS FROM A CASE OF BACTERIAL ENDOCARDITIS*

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A PREVIOUSLY undescribed bacillus was obtained on three different occasions from the blood stream of a patient with bacterial endocarditis. Its source and portal of entry are not known.

CASE HISTORY

The patient was a thirty-five-year-old woman when first seen in the clinic. At the age of thirteen years she had tonsillitis and sore throats. These were followed by an attack of swelling, tenderness, and redness of several joints. Ascites and edema appeared after the acute attack of rheumatism. She was told she had heart disease. At the age of sixteen her tonsils were removed. She married at twenty-four years of age and went through two pregnancies, at twenty-six and thirty years, without difficulty. The patient gained 15 pounds during the latter pregnancy and then voluntarily reduced her weight to 104 pounds. She never felt strong again and began to have mild dypsnea on exertion. During the five months prior to her admission to Billings Hospital she had several bouts of perspiration associated with sensations of chilliness. She had a cough that was productive of mucopurulent sputum for three weeks just before hospitalization. Although the patient was in bed she experienced dull aching pain over the precordium for a few days before admission. She also had pain in the right costovertebral angle and described her urine as being "unusually dark." Several weeks before she was first seen a finger tip was tender and swollen.

The admission examination revealed a sick-looking, undernourished, dyspneic woman whose temperature was 100.4° F. The pulse rate was 108/min., the respiratory rate was 20/min. and the blood pressure was 112/50. The diastolic range was poorly defined and may have been lower. Other salient features of the examination included scattered petechiae in the skin, a large, hyperactive heart and a large firm palpable spleen. The precordium heaved synchronously with the heart beat. A systolic thrill was palpable over the apex of the heart. The apical thrust was diffuse and prominent. The second sound was not heard at the aortic region. A loud systolic murmur was audible over the entire precordium. A short, faint diastolic murmur was detected over the aortic region replacing the second sound. The pharynx was not inflamed.

The laboratory procedures gave the following results. On May 8, May 14, and August 5, 1936, blood cultures were taken. On each occasion a bacillus was obtained in pure culture. Agglutinations with the patient's serum against the typhoid, paratyphoid and *B. abortus* groups were positive only with the *E. typhosus* antigen in a dilution of 1/20. The others were entirely negative.

Stereoscopic x-rays of the chest showed the heart to be "slightly less than 65 per cent over-size by film tracing." Early congestive changes were reported in both lungs.

Besides a tachycardia of 100/min. an electrocardiogram showed broad P-waves in all leads and T_2 to be low in amplitude.

The blood Kahn was weakly positive and the Wassermann was negative.

From May 8 to May 15, 1936, while the patient was in the hospital, the leucocyte count varied from 9,100 to 11,100 cell/c.mm. The differential count showed a mild absolute increase in polymorphonuclear leucocytes. A moderate secondary anemia was present at all times.

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The urinary findings were usually not abnormal. On one occasion a trace of albumin was found. Rarely red and white cells appeared in the sediment.

The patient was last seen in August of 1936, three months after the first two blood cultures were obtained. The third culture was taken at that time. She died about a month later at home. An autopsy was not obtained.

It is apparent that this is a case of bacterial endocarditis in a person who had rheumatic fever in childhood. The myocardium was strong enough to carry through two pregnancies without incident. From the story one can judge the duration of the bacterial endocarditis to have been at least nine months. Most likely the mitral and aortic valves were ulcerated.

Description of Organism.—The bacillus obtained from this case grew out in pure culture on all three attempts. The first and the last were three months apart. No other organisms or pleomorphic variants were discovered. The organism was a gram-negative bacillus with pointed ends. The bacilli had an average length of 2μ and a thickness of 0.28μ. They varied slightly in length and very little in width. Stained by the Gram method barring was some-

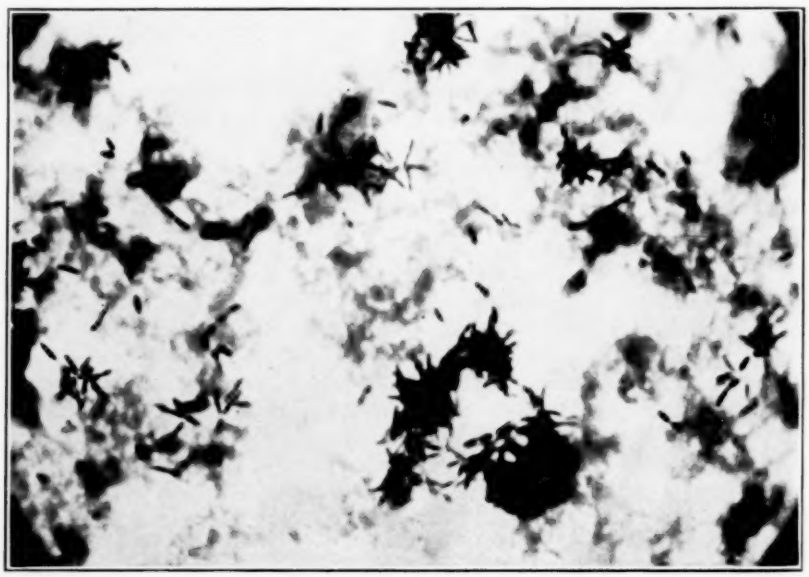

Fig. 1.—A smear from a broth culture. Stained by the Gram method. Magnification ×2300.

times seen; also bipolar forms appeared in the same smear. The fresh twenty-four-hour cultures taken from the patient tended to be slightly gram-positive but gentian violet was not retained in cultures older than twenty-four hours. Subcultures were invariably gram-negative. The bacilli tended to form short filaments which had in places gram-positive spore-like ends. Occasionally an isolated gram-positive organism appeared in a smear in the form of a lancet-shaped diplococcus. The bacillus was not acid fast. In dextrose broth it grew as a granular deposit. It grew slowly at first on beef infusion agar but readily after several subcultures on that media. The colonies were smooth and round. They resembled diphtheria colonies in size, shape, and color. On chocolate agar, they were discrete yellowish colonies similar to those grown on beef infusion agar except for the slight increase in pigmentation. Although subcultures were not at first successful on litmus lactose and sheep blood, beef extract media, the organisms grew out after several trials. The organisms growing in broth were not motile. No indol was formed. Gelatin was not liquefied. The following sugars were fermented with production of acid and gas: dextrose, mannite, maltose, and sorbital. These were not affected: lactose, sucrose, and galactose. Acid without gas was formed in trehalose. No other sugars were used.

The organism was agglutinated by the patient's serum in a dilution of 1:5120. In three normal controls, slight, poorly defined agglutination was noted. The organism was not pathogenic for mice, rabbits, or guinea pigs.

The organism ceased to grow after several subcultures and was lost. From the above findings it is likely that this organism falls into the order Actinomycetales. It does not parallel any other particular species.

DISCUSSION

At least 26 different species of bacteria have been isolated in pure culture from the blood streams of patients with endocarditis. The disease has been described in association with virus disease.[1] The usual habitats of these organisms include all recognized portals of entry of the body. It is not surprising to encounter an unusual invader in the blood stream of individuals with bacterial endocarditis.

Reports have appeared in which diphtheroids were grown from the blood of patients with bacterial endocarditis and green forming streptococci were recovered from the vegetations in these individuals after death.[2] Cases and experiments have been described in which diphtheroid organisms obtained during life from cases of bacterial endocarditis were shown to be related to green forming streptococci as mutants.[3-5] When a pure culture of bacteria has been isolated on more than one occasion from an individual, it has invariably proved to consist in organisms responsible for the valvular infection. It is suggestive that the bacillus presented here was likewise the primary agent of the terminal disease.

CONCLUSIONS

A bacillus that fits best into the order Actinomycetales was discovered by culture in the blood of an individual with bacterial endocarditis. A description of this organism was not found elsewhere.

NOTE: Advice and assistance were gratefully accepted from Dr. George F. Dick and Miss Helen Van Sant.

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THE EFFECT OF SALTS OF CITRIC ACID ON SERUM CALCIUM IN THE ALBINO RAT*

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SINCE sodium citrate has been shown to be lethal to the spirochetes of the mouth[1] and has been used successfully in the local treatment of Vincent's infection of the mouth and pharynx, it has seemed of interest to observe the effects on serum calcium of three salts of citric acid.

The salts studied are trisodium citrate, ammonium hydrogen citrate, and ferric ammonium citrate.

Albino rats were used for the calcium studies because of the convenience in handling this species and because white rats were made available to us through the courtesy of the Anatomy Department of Emory University Medical School. The animals were descended from a strain heterogeneous in origin but inbred for several years in the Anatomy Department laboratory. A considerable degree of uniformity has been attained in size and longevity; the mortality rate is low and fertility is high. Animals used in these experiments were from eighteen to thirty months in age and weighed from 200 to 300 gm. Both sexes were used indiscriminately.

Injections were made under ether anesthesia via a tail vein with a graduated tuberculin syringe and a twenty-eight gauge needle, as rapidly as a smooth, steady flow into the vein could be induced. The salts were administered in the form of aqueous solutions usually of 20 per cent strength. The minimum lethal dose was roughly determined for each salt, and in each case was seen to vary over a short range of dosage in different individuals. The cause of this variation was partly dependent on individual physiologic variations among the rats but also may have been affected by technical variations which were not eliminated, since the calcium levels found in each set of experiments were consistent and the data supported the assumption that in all cases the conditions of the experiments were sufficiently uniform for our purposes. Technical variations which may have affected the amount of the minimum lethal dose include the time consumed in making the injection, the possible leakage from the vein into the tissues and the extent of dilution of the blood where different amounts of the solution were given to rats of different weights. The findings with reference to the minimum lethal dose are presented in Table I. It will be seen that this dosage covers a not very wide range of concentrations for all three salts, under the conditions of our experiments.

Blood was drawn from the heart under ether with an ordinary 1 or 5 c.c. syringe and a 2 inch hypodermic needle. Serum was obtained in the usual

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way. The time which elapsed between the completion of the injection and the drawing of blood varied from ten to forty minutes. Within this range the calcium level had no relation to the time interval, low and high values for serum calcium being found at either extreme.

TABLE I

MINIMUM LETHAL INTRAVENOUS DOSE OF CITRATES FOR RATS

SALT	MG. SALT/GM. RAT	EFFECT ON ANIMAL
Sodium citrate	1.0-1.5	Tetany for about ½ hour; death
Ammonium citrate	1.9-2.0	Slight tetany with apparent recovery after about 10 min.; death after about three hours.
Ferric ammonium citrate	1.0	No tetany; death after about one hour.

For the determination of serum calcium the micromethod of Hirschfelder and Serles[2] was used. This method is a modification of the well known Kramer-Tisdall method. The micromodification requires only 0.2 c.c. of serum and quantities of reagents are correspondingly reduced. Practice is required in observing the end point of the titration with diluted potassium permanganate (0.001N), and the results here obtained are believed to exceed somewhat the true values owing to error in titration. This error, however, is believed to be consistent throughout the determinations, since all were made by one individual and good agreement was obtained between duplicate titrations.

The results of the work on serum calcium are presented in Table II.

TABLE II*

SERUM CALCIUM IN NORMAL RATS AND IN RATS INJECTED WITH SALTS OF CITRIC ACID

NO. OF ANIMALS	HISTORY	SERUM CALCIUM (RANGES OF VALUES FOUND)	SERUM CALCIUM (AVERAGE)	COMMENT
5	Normal	11.0-15.9	12.6	
3	3-5 days after injection	11.3-15.5	13.5	2 rats injected with sodium salt; 1 rat injected with ferric salt. No tetany at any time.
7	Sodium citrate 0.83-1.0 mg./gm. rat	4.0-11.5	7.2	Marked tetany prolonged beyond 10 min. Death if lasts 30 minutes.
5	Ferric ammon. citr. 1 mg./gm. rat	11.3- 4.8	13.3	No tetany.
6	Ammonium citr. 0.98-1.8 mg./gm. rat	13.9-15.1	14.2	Slight tetany with recovery within 10 minutes.

*Serum calcium expressed as mg. per 100 c.c. serum, i.e. as mg. per cent.

The normal values found are somewhat higher than the figures for the normal reported by Shohl and coworkers[3, 4] in two papers, whose normal range was from 10.4 to 13.8 mg. per cent. McClow and Koch[5] report an even lower range for normal serum calcium in rats, i.e., 4.7 to 11.1 mg. per cent. If the results cited from both groups of workers are accepted, it is seen that in the present investigation only one instance of a calcium value below the lower limit of the normal range was observed. However, the calcium values found and the effect of the salt on voluntary muscles was correlated here, where comparable quantities of each salt were administered.

From the above data of Table II, it is seen that there is some relation between the onset of tetany and a lowered serum calcium level, although a low calcium level is not always seen where tetany occurs. In these experiments, a drop in the serum calcium below 11 mg. per cent, following the injection of sodium citrate, was accompanied by marked tetany. After ammonium citrate, slight attacks of tetany generally occurred with no fall in serum calcium, but the attacks were of short duration with recovery to normal movement even when death eventually ensued. Ferric ammonium citrate induced no fall in serum calcium and no signs of tetany. Shohl and coworkers[6] have observed an association of tetany in rats with the reaction of the salt mixture given with synthetic diets, where a neutral diet was always accompanied by tetany and an average serum calcium level of 11.9 mg. per cent. Alkaline diets were only occasionally associated with tetany, although serum calcium fell to values between 7.7 and 8.2 mg. per cent. With acid diets, often associated with rickets, tetany was never observed, although serum calcium had a value of 9.0 to 9.8 mg. per cent. The value of the Ca:P ratio may be involved in the production of some types of tetany and the value of serum calcium alone cannot be relied on as an indication of the imminence or improbability of an attack of tetany, although tetany probably should be expected if serum calcium falls to a sufficiently low level, regardless of blood phosphorus.

SUMMARY

1. Normal serum calcium in white rats was found to range between 11.0 to 15.9 mg. per cent.

2. Three salts of citric acid were studied as to the effect on serum calcium and the onset of tetany after intravenous injection in white rats.

3. Serum calcium was markedly lowered after injection of sodium citrate but showed no deviation from the normal range of concentration after injection of ferric ammonium citrate and of ammonium citrate.

4. Tetany followed injection of sodium citrate exceeding 0.9 mg. per gm. rat; slight tetany, invariably followed by recovery of normal movement, followed intravenous doses of ammonium citrate exceeding 1 mg. per gm. rat; injection of ferric ammonium citrate was never observed to produce signs of tetany.

5. The minimum lethal dose of each salt for the albino rat was roughly determined.

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ONSET AND COURSE OF EPIDEMIC MENINGITIS AS RELATED TO FLUCTUATIONS IN TEMPERATURE AND BAROMETRIC PRESSURE*

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SEASONAL variations in the incidence of diseases transmitted through the medium of the upper respiratory tract with concentration of cases during the colder months has long been appreciated and taken cognizance of. It was deemed advisable to subject this gross accepted fact to more detailed analysis. With this in view a daily study of weather conditions was made and correlated with the incidence of epidemic meningitis in Sangamon County, Illinois during 1935.

In order to logically lead up to the presentation which is to follow, it will be necessary to state some of the salient features relevant to the transmission of the meningococcus and its mode of invasion of the body.

It is well known that persons associated with the patient rarely acquire the disease. These people, however, frequently become carriers and disseminate the infective agent to others. Healthy passive carriers outnumber the cases of epidemic meningitis 10 or 30 to 1. It has been found that the percentage of carriers varies from 1 per cent in individuals who have had no known contact with cases to 80 per cent among intimate contacts under military conditions. The healthy passive carrier, therefore, is considered to be the main source of infection. The carrier rate is higher in the cold months than in the summer.

The infection itself is divided into three stages: the first, a localization in the upper air passages, commonly the nasopharynx; the second, an invasion of the blood stream; the third, metastatic localization commonly in the meninges, but also in the joints, pericardium, endocardium, lungs, skin and other foci. It of course may not be possible to distinguish three such stages in every case.

It has often been a matter of conjecture as to why so many people may harbor the meningococcus in the nasopharynx and so few develop the clinical disease. This question would be directly referable to the invasive powers of the organism, tissue permeability, and resistance of the host. It is not the purpose of this paper to attempt to explain all the factors but merely to present evidence as to one possible influential condition favoring the invasion of body tissue by the meningococcus.

Petersen[1] has previously shown the significance of the passage of atmospheric fronts for the penetration of the meningococcus. Thus with a polar front (or the unusual development of a tropical front), the individual develops a distinct "pressor episode" with general or localized areas of vascular spasm and anoxemia. This period of vascular spasm is followed by either general or local stimulation of the organism. During this latter phase bacteria are more likely to

*From the Director, Champaign-Urbana Public Health District.
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pass the mucous membranes, or, if circulating in the blood stream, may become localized in regions previously anoxemic because the vascular endothelium has here become more adhesive. Regions of terminal vascularization—the anoxemia having been more pronounced—will be seemingly selective in their greater disposition to infection under such conditions.

In studying the possible relation of meteorologic alterations to tissue invasion and localization of the meningococcus, the data for analysis were obtained from the epidemiologic cards available in the Division of Communicable Diseases of the Illinois Department of Public Health. The cases of epidemic meningitis studied were those occurring in Sangamon County during the year 1935.

A meteorograph was prepared for the periods in which cases occurred. The solid line in the middle of the graph indicates the mean daily barometric

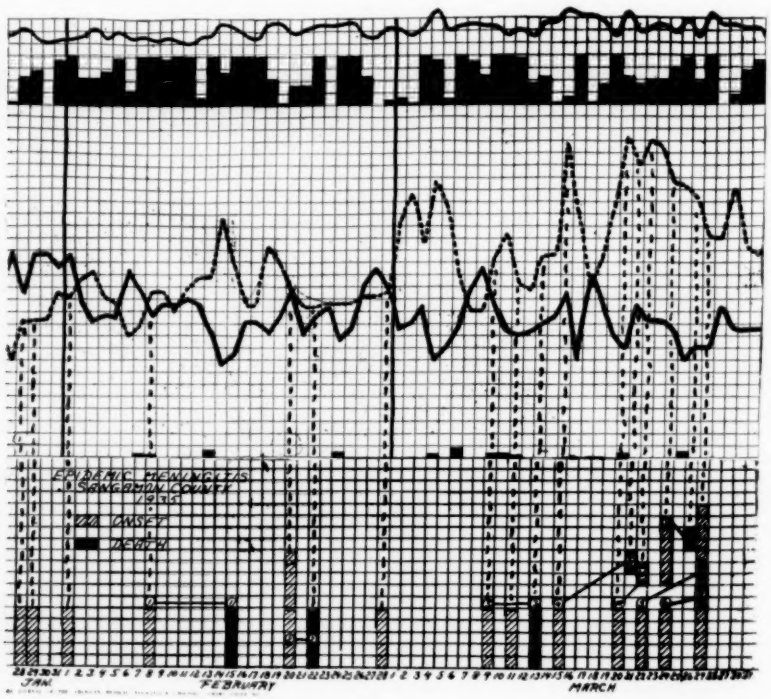

Fig. 1.

pressure, and the dotted line the mean daily temperature. The solid areas at the top signify percentage of cloudiness and the continuous solid line at the extreme top, the daily wind velocity. The date of onset of cases is indicated by the striated blocks, and the date of death, where fatal issue resulted, by solid blocks. Each figure represents one case. The date of onset and death in the fatal cases is signified by corresponding numbers connected with a solid straight line. The graphs and their interpretation are now presented.

The cases of January 28 and 29 both follow immediately in the wake of a polar crest that reached its maximum on January 27.

A case occurs on February 1 during a minor polar infall but the general tendency is for rising temperatures.

The first fatal case has its onset on February 8, again immediately in the wake of a polar crest which occurred on February 6 and 7. The patient dies on February 15 at the beginning of a very sharp polar front.

Two cases have their onset on February 20 during the next succeeding front and one of these dies on February 22 at the polar infall initiated on February 20.

One case occurs on February 28 at a tropical front but actually on the day following a great barometric crest.

A case (3) occurs on February 9 at a period that is quite similar to the preceding and this patient dies one day after the succeeding polar episode of March 12.

One case during the stormy period of March 11. This is coincident with a polar infall.

A case (4) occurs with a barometric crest on March 5 and dies with the polar front of March 21.

Fig. 2.

A case (4A) occurs at the tropical period of March 20 and dies immediately in the wake of a barometric crest that was associated with death in the preceding case.

A case (5) originates at the same time and the patient dies during the polar episode that began on March 23 and extended to March 28.

Two cases (6 and 7) begin at the polar front on March 24. Patient (Case 7) dies on March 26 and another patient (Case 6) dies on March 27 as there is a renewal of the polar effect.

Another case is reported with the polar front of March 27.

Two cases on April 7 and 8, respectively, at the time of a stormy period associated with falling temperatures, falling barometer and heavy precipitation (partially occluded cyclone).

Another case occurs with the polar infall of April 15.

A case occurs during a polar episode evident during the time when there was rising temperatures.

A case occurs with the tropical crest of May 22.

A case occurs immediately in the wake of the barometric crest of May 25.

A final case occurs that is coincident with the polar infall of May 29.

Case 8 begins with the tropical crest of November 9, but at a time when the barometric curve indicated a coincident polar effect. The patient dies following the polar infall of November 14.

A case occurs at a tropical period of November 20. This is one day after an extension of the barometric high which reached its crest on the sixteenth of November.

A case occurs at a polar front on November 22.

Two cases occur with the tropical fronts of November 25 and 27, the first immediately after the great barometric crest of November 24, and the second with the distinct extension of the barometric crest evident in the graph.

A case occurs immediately after the barometric crest of the fourth of December.

A case occurs at the polar crest of December 11.

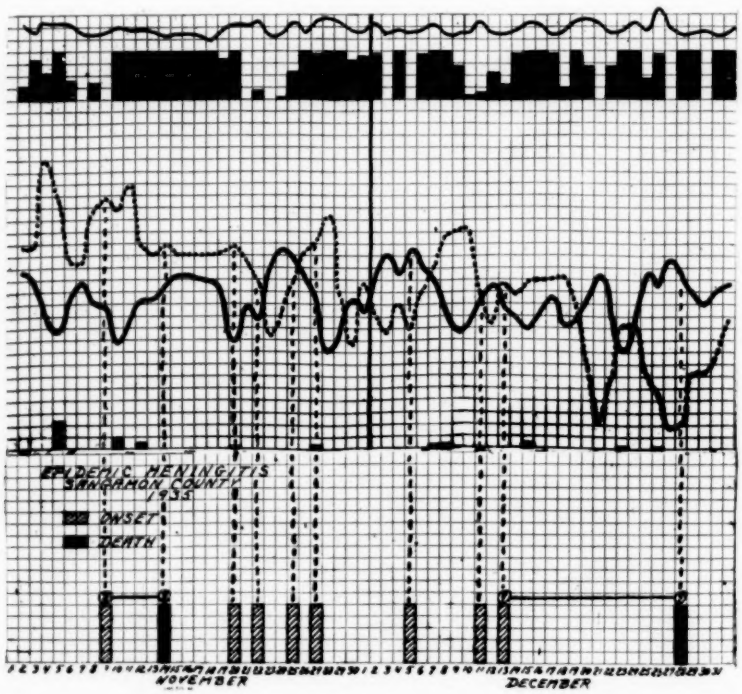

Fig. 3.

Case 9 begins immediately in the wake of this same crest and the patient dies with the final cold wave of the month.

It is probable that bacterial penetration of tissue is of common occurrence. Unless the organism concerned is of high virulency or the local resistance is low, there probably results nothing more than reticulo-endothelial fixation of the invader with subsequent destruction. It may be assumed that in some instances the organism so fixed is not destroyed, but remains quiescent. If such be the case, the occurrence of a sudden pressor episode, meteorologic or otherwise, may cause the mobilization[2] of the fixed bacteria and their being thrown into the circulation.

The effect of the pressor episode correlates rather nicely with the three stages previously described in the development of epidemic meningitis. First we have

the deposition of the bacterium in the nasopharynx as a result of contact with case or carrier. Penetration of tissue must be assumed to exist, markedly enhanced after the pressor episode. The degree of such an effect will vary with the individual. In some cases there will be fixation and destruction of the bacterium. In others there will be temporary quiescence. With an additional pressor effect, mobilization can occur, associated with dissemination through the medium of the circulation. This is in accord with our conception of a stage of bacteremia.

Since a reflex vasoconstriction occurs in the brain following closely the similar phenomenon observed associated with the chilling of skin and mucous membrane, with associated anoxemia and increased adhesiveness of the vascular endothelium, subsequent deposits of the meningococcus in the meninges, from the circulation, would be favored.

SUMMARY

The study of the onset of meningitis in Sangamon County, Illinois during the year 1935 has confirmed the evidence previously presented by Petersen concerning the significance of the passage of atmospheric fronts for the invasion of the body by the meningococcus.

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LABORATORY METHODS

HOT AIR STERILIZATION AND ITS PRACTICAL APPLICATION IN BACTERIOLOGIC LABORATORIES*

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THE difficulties in obtaining good results in bacteriologic work in laboratories have taught us to lay particular stress on thorough sterilization of the glassware intended for experimental purposes. Various methods for sterilization have been employed, yet not one of them has proved to be completely satisfactory.

To render the glassware sterile means to kill all bacteria present in the glassware. Bacteria can be killed by diverse agents. All agents must act upon bacteria by chemical or physical processes in such a way as to render them dead. The bacterial cell is considered dead when there is a permanent cessation of all the anabolic and catabolic changes which under normal conditions manifest themselves in it, followed by a decomposition of the resultant fixed substances. It therefore constitutes such a state where neither the growth, nor the multiplication of the bacteria is any more possible.

The most valuable agent for the destruction of bacteria is heat. There is a marked difference between dry and moist heat. This difference is of great practical value, since wet air (water) has a higher potency for killing bacteria resistant to heat in a shorter period of time. This is very important for disinfecting purposes.

The bactericidal action of dry heat takes place at very high temperatures. Bigelow and Esty have demonstrated that the destruction of spores by heat is not instantaneous but a gradual process during which the spores are progressively injured and finally destroyed. An organism at the temperature above the maximum for its growth cannot survive, and the higher the temperature to which it is exposed, the sooner will it be killed.

Lister in 1878 devised the hot air sterilizer in order to destroy all organisms present in dust, air, water used for washing, and in the packing material of glassware. Since among the organisms found in the air, dust, packings, etc., there are spore formers, it is necessary for the proper sterilization to destroy not only the nonspore-bearing bacteria but also the spores which have, so to say, a protective function. To accomplish their death it becomes essential to study the nature of the spores, their environmental conditions, normal habitat, and the optimal temperature and time for their growth and destruction.

Reichenbach in 1908 demonstrated that various spores show a variable degree of resistance to heat and that the survival of certain spores at tempera-

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tures at which others are destroyed can be traced to individual resistance. The law of mass action cannot be applied to spores, since the destruction of spores in different species takes place according to different laws.

Besides the variation in resistance of spores of different species, the resistance may also differ in the various developmental stages of the spores of the same species, as well as with various environmental conditions. Old spores are less resistant to heat than fully developed young ones. Bigelow found that young spores about one month old have the highest thermal resistance. On the other hand, the vegetative forms are less resistant to heat than the fully developed spores. Again, dry spores manifest a slight increase in heat resistance as a result of dehydration, while the same spores in a moist condition stored exactly the same length of time would exhibit essentially the resistance of young spores. Pasteur has demonstrated that spores remain alive in a dry form for twelve or more years.

Various theories have been advanced to explain this high resistance of spores to heat. At present it seems reasonable to assume that it is due to: (1) the spore membrane which acts as a protective structure; (2) the low water and salt content of its protoplasm as compared with that of the vegetative cell.

The abundance of spores is not always an indication of the presence of highly resistant forms. Nevertheless, a larger amount of spores will require a higher temperature and a longer period of exposure for them to be killed. It has long been recognized that a very definite relationship exists between the time necessary to destroy any given suspension of organisms and the temperature to which the suspension must be exposed. Williams, therefore, introduced the term "thermal death time" in preference to the older term "thermal death point."

The behavior of spores when subjected to heat has been and is continued to be studied by numerous investigators. Tyndall in 1877 demonstrated that the spores of certain bacteria have two phases, one being thermolabile and destroyed at 100° C. in five minutes, and the other, what he calls "the germ of the bacterium" being thermoresistant to an almost incredible extent. Ferdinand Cohn in the same year arrived at the same conclusions by different methods. In order to kill the former and overcome the latter, Tyndall developed a method of fractional sterilization by discontinuous heating known as tyndallization.

Another interesting phenomenon has been observed in the so-called "skip-stop" stages. These skip-stops appear during or after three months of incubation and are believed to be the result of an uneven distribution of the spores. This phenomenon assumes special significance in sterilization and can be eliminated by a sufficiently prolonged incubation and by a careful preparation of a uniform suspension with an even distribution of the organisms.

The author of this paper has undertaken an extensive study of hot air (dry) sterilization, dealing with the processes taking place in the hot air sterilizer, and also of the heat resistance of *Bacillus subtilis*, since it is frequently found in the glassware and is believed to be the strongest or most stubborn organism to heat destruction. This paper is divided into two parts: the first part dealing with the functions and sterilization properties of the sterilizer,

and the second part with the behavior of *Bacillus subtilis*, when submitted to dry heat. For this purpose a series of tests over a period of ten months was performed and the results recorded in the charts and tables.

The hot air sterilizer used for these tests is constructed as follows: It is made of supermetal (galvanealed iron) and is provided with three metal walls. Between the outer and the second wall which form one side of the flue, there is a layer of one inch asbestos insulation; then an inner metal wall forming the chamber and the other side of flue. Beneath the oven are mounted three gas burners. The compartment is perforated on top and bottom. Flues are arranged at sides, back, and top, and dampers are provided. Gas burners are baffled, some of the products of combustion passing around the sterilizing chamber, and others passing through it, providing a forced air circulation necessary for an even temperature. The oven is fitted with an automatic thermostatic control with an expansible element extending half way across the chamber at the top. The individual burners are equipped with gas cocks and air mixers, but all are controlled by the thermostat. The top of the sterilizer is fitted with three tubulatures where thermometers are inserted. The forced air circulation distributes the temperature. In order to find out whether the temperature is evenly distributed to render all glassware placed in the sterilizer sterile, minimum and maximum thermometers were placed inside to depict the various temperatures at various angles of the sterilizer. The sterilizer thus constructed was set into operation and an extensive study of the time-temperature relation was made. Observations were first made with an unloaded sterilizer, the initial temperature being approximately 20° C. (Chart 1). Readings were taken at five-minute intervals. At the end of two hours the gas burners were turned off and the thermometers registered the following temperatures: the right one 165°, the left one 180°, and the center one 170° C.; the minimum and maximum thermometers, 175° C. Therefore with the sterilizer empty, the inside temperature rapidly rises, while in the presence of a load, as soon as it reaches 150°, the thermostat comes into play by cutting down the flame of the burners.

The experiment was then repeated with a moderate load (Chart 2) consisting of 4 square crates of Petri dishes, 6 crates of test tubes, 2 crates of bottles and packages of pipettes wrapped in paper. The burners were lit at 9:45 A.M. with the initial temperature of about 20°, and at the end of two hours the gas was turned off. The right thermometer registered 172°, the left 175° and the center 160° with the inside temperature of 173° C.

Thus with this moderate load it took forty minutes for the load temperature to reach 153°, the temperature at which suitable sterilization begins. It is also important to note that with this load both the inside and outside temperatures closely approximate each other throughout the length of the experiment. Therefore, the outer thermometers could be used as a fair index of the inside temperature, eliminating the necessity of frequent opening of the sterilizer while it is in process of sterilization.

The third test was performed on an overloaded sterilizer (Chart 3). The load consisted of ten large crates of Petri dishes; 2 crates of test tubes; of many paper wrapped articles. The upper shelf of the sterilizer was over-

loaded with packages of pipettes, wrapped in paper, and piled up in bundles. The sterilizer was started at 10:25 A.M. At 11:10 A.M., i.e., at the end of forty-five minutes of operation, when the outside temperature reached 155° at the right thermometer, 163° at the left one, and 150° at the center thermometer,

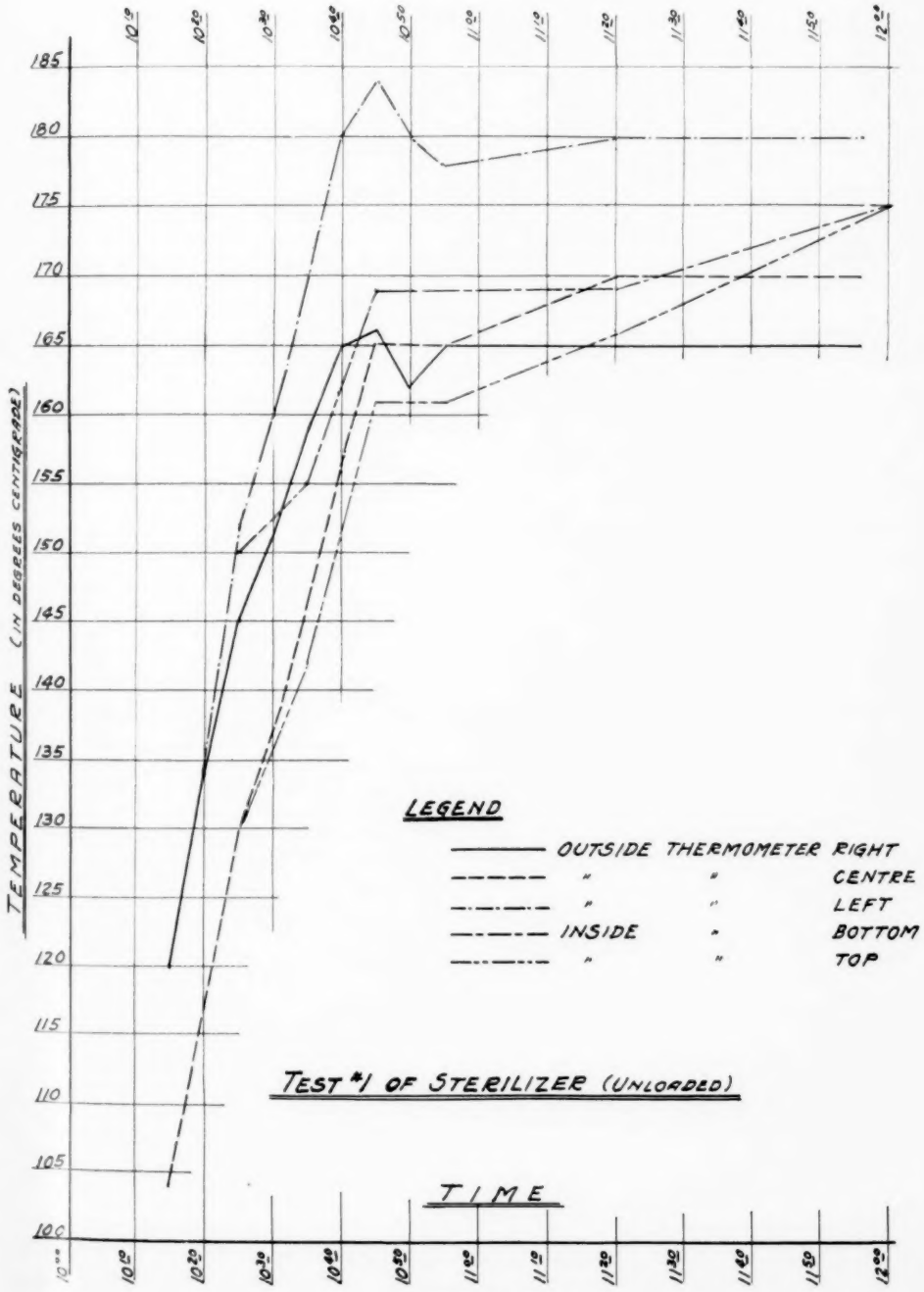

Chart 1.

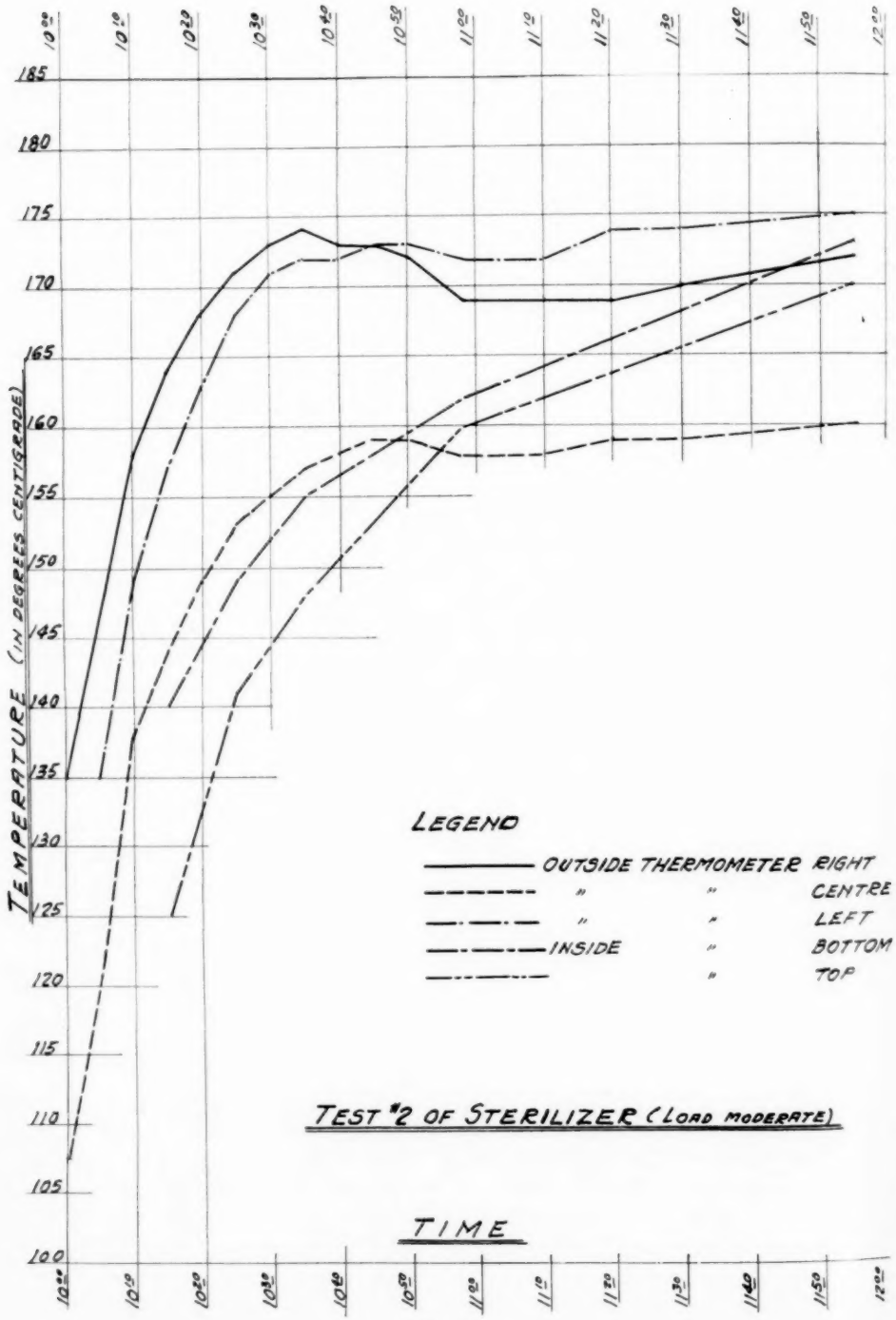
TEMPERATURE (IN DEGREES CENTIGRADE)
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
10 00
10 10
10 20
10 30
10 40
10 50
11 00
11 10
11 20
11 30
11 40
11 50
12 00
LEGEND
OUTSIDE THERMOMETER RIGHT
" " CENTRE
" " LEFT
INSIDE " BOTTOM
" TOP
TEST #2 OF STERILIZER (LOAD MODERATE)
TIME

Chart 2.

the inside thermometers registered 125° between the Petri dishes and 105° in the bundles of the pipettes (paper). At the end of two and one-fourth hours the gas burners were turned off and the following temperatures found: at the

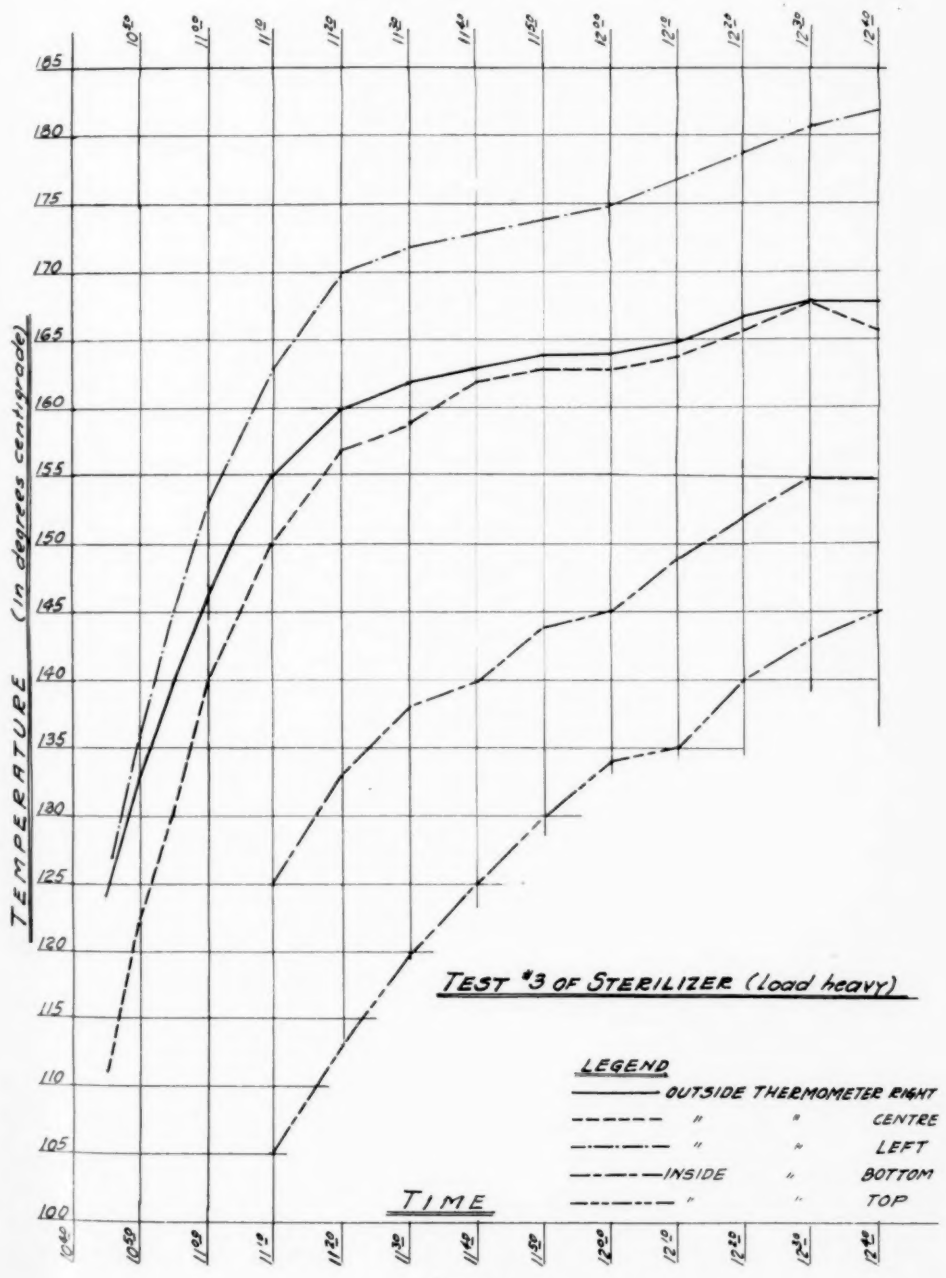

Chart 3.

right 168°, left 182°, and the center 166°. The inside temperatures were 155° between the crates, 145° within the paper wrappings of the pipettes, and 150° between the Petri dishes.

It is evident from these observations that with an overload the time of sterilization must be considerably prolonged, and even when the center thermometer registers 150° C. or thereabout, the inside temperature still remains

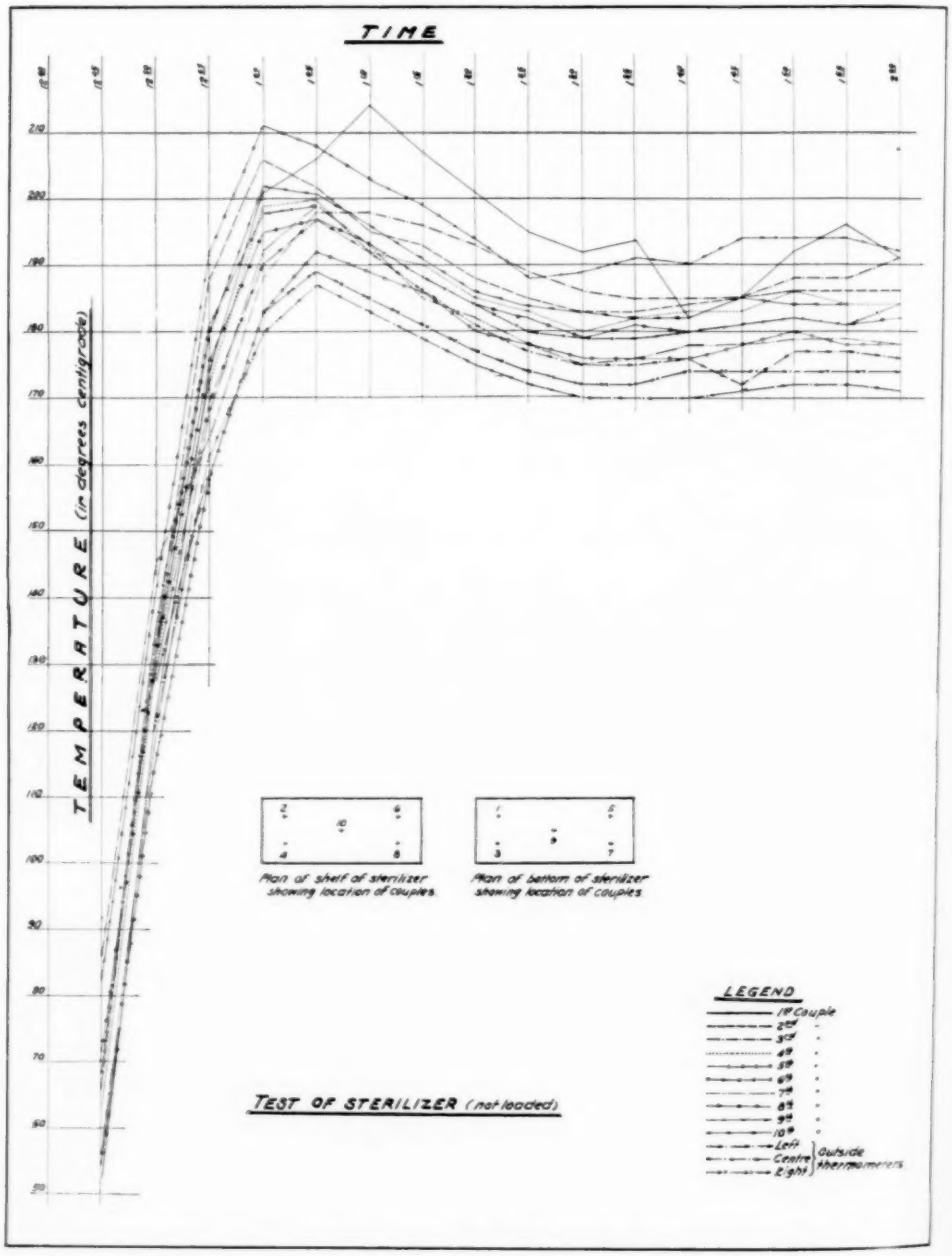
TIME
TEMPERATURE (in degrees centigrade)
Plan of shelf of sterilizer showing location of couples.
Plan of bottom of sterilizer showing location of couples.
LEGEND
TEST OF STERILIZER (not loaded)

Chart 4.

for a long time below this point. Besides, there is also a difference in the temperature in various points of the sterilizer as well as within the various containers of the articles to be sterilized. As a control, a series of similar tests

with an unloaded and loaded sterilizer was run. In addition to the minimum and maximum thermometers, the temperatures were checked also by thermo-

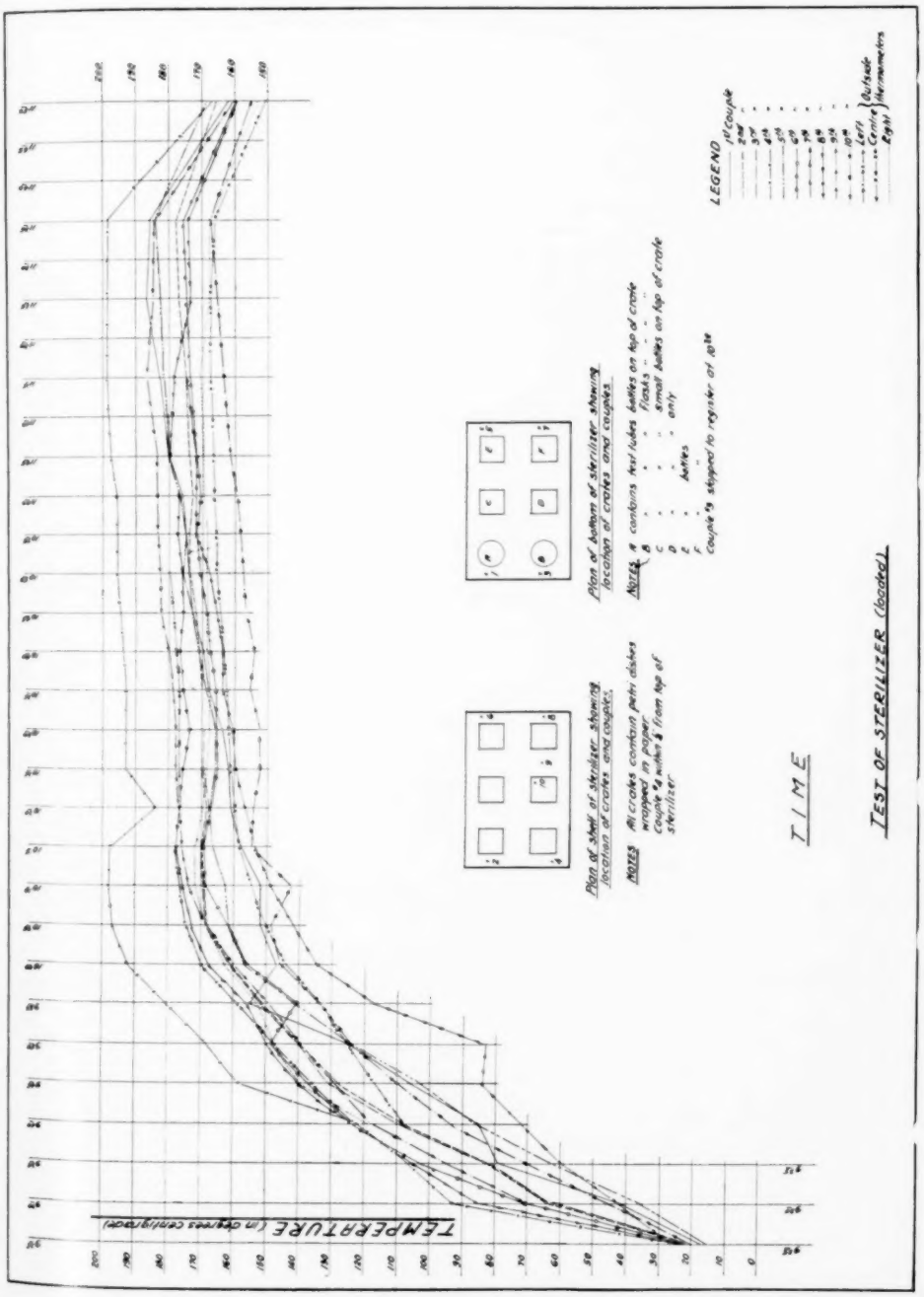
TEST OF STERILIZER (Loaded)
TEMPERATURE (in degrees centigrade)
TIME
LEGEND
Plan of bottom of sterilizer showing location of crates and couples
Plan of shelf of sterilizer showing location of crates and couples

Chart 5.

couples, since they are more sensitive to changes in temperatures. The results are recorded in Charts 4 and 5.

The findings of these experiments were as follows: if the sterilizer was unloaded, it maintained an approximately even temperature, but when the sterilizer was loaded, there occurred two temperatures, one being the air temperature and the other the load temperature.

The above-mentioned experiments have shown that in order to assure proper results the following principles must be adhered to in operating this sterilizer.

TABLE I*

140° C. ONE HOUR VARIOUS ORGANISMS THERMAL DEATH TIME

TYPE OF GLASSWARE USED IN TEST	MATERIAL SEEDED	POSITION OF GLASSWARE IN RELATION TO LOAD	CHARACTER OF LOAD	POSITION OF LOAD IN STERILIZER	FINDINGS
8 ounce bottle	Subtilis X	In crate	Crate of T. B. tubes	Back top, right	Free spores
Test tube	Dust	Attached to crate	Crate of centrifuge tubes	Front bottom, center	Long chains
Petri dish	Dirt A	On top of crate	Crate of bottles	Front, top shelf	Free spores
4 ounce bottle	Subtilis S	Between crate and wall of sterilizer	Crate of Petries	Center, bottom	Free spores
Petri dish	Staphylococcus	On bottom of crate of tubes	Crate of potato tubes	Front, bottom	No growth
125 c.c. flask	Dirt B	In crate	Crate of small bottles	Center, top	Short bacilli
Petri dish	Subtilis X	Center of crate	Crate of Petries	Front, bottom	Free spores
Potato tube	Subtilis S	In crate	Crate of inverted tubes	Front top, left	Spores
Test tube	Dirt A	In rack	Rack of Smith fermentation tubes	Center, bottom	Cells in pairs
Petri dish	Staphylococcus	Between pipettes	Packages of pipettes	Center, top	No growth

*NOTES: Subtilis X, pure culture isolated from hay.
Subtilis S, stock culture used in our laboratory.
Dirt A, from our animal house.
Dirt B, from the street.
Dust, collected in the laboratory.
Staphylococcus, stock culture.

These various types of glassware with planted organisms from above-mentioned material were sterilized at 140° C. for one hour. Extract broth and extract agar were added and incubated at 37° C. for twenty-four and forty-eight hours. All the bacteria survived, except staphylococcus cultures which were killed.

A. *Regarding the Loading of the Sterilizer.*—The sterilizer must not be overloaded. Only a moderate load of approximately 10 crates or its equivalent may be placed in it, so as to permit a free passage of heated air around, under and through the articles. Paper wrappings or packings are to be avoided as much as possible, since paper forms a dead wall and interferes with the penetration of the hot air into the load.

B. *Regarding the Spacing of the Material.*—The load in the sterilizer should be arranged so that the articles should not rest on each other, nor touch the sides or back of the sterilizer. Pipettes are best placed on the upper shelves of the sterilizer in order to avoid burning of the paper wrappings. They should be scattered loosely and not piled up in bundles, so as to assure free passage of air between the packages. On the other hand, pipettes in metal containers may be placed on the bottom, or any part of the sterilizer, since metal being

a good conductor of heat will not disturb the process of sterilization. Special care should be taken in sterilizing Petri dishes. It is bad practice to place the Petri dishes in a crate lined with paper. Instead they are to be placed in crates and only the top of the crates covered with paper.

C. *Regarding Operation of the Sterilizer.*—When the sterilizer is started and the gas burners are properly lit, the thermometers will begin to register the inside temperatures, the center one carrying the load temperature, and

TABLE II*

150° C. FIFTEEN MINUTES BACILLUS SUBTILIS G. (HAY) THERMAL DEATH TIME

TUBE	POSITION OF TUBE IN RELATION TO LOAD	CHARACTER OF LOAD IN STERILIZER	POSITION OF LOAD IN STERILIZER	FINDINGS	
				MACROSCOPIC	MICROSCOPIC
1	In crate	Crate of bottles	Center, bottom	Granular pp.	Long chain formation
2	Under paper cover in crate	Crate of Petri dishes	Center, front	Heavy pellicle	Long chains
3	On top of crate	Crate of potato tubes	Center, bottom	Cloudy	Long bacilli
4	Tied to crate	Crate of Petri dishes	Front, upper right	Cloudy	Short bacilli
5	On top of crate. Tube wrapped in newspaper	Crate of bottles	Center, bottom	Slightly cloudy	Bacilli and threads
6	Attached to crate, 4 inches from bottom	Crate potato tubes	Center, bottom	Cloudy	Cells in pairs rounded edges
7	Attached to crate, near wall of sterilizer	Crate bottles	Front left, bottom	Granular pp.	Short bacilli
8	Lying on bottom of crate wrapped in paper	Square crate tubes	Front, right	Slightly cloudy	Long chains
9	Top of crate	Crate of Petri dishes	Rear left, top	Cloudy	Short bacilli
10	On crate of bottles	Crate of bottles	Rear left, bottom	Pellicle	Long chains
11	Between wall of sterilizer	Crate of Petri dishes	Upper left, top	Cloudy	Cells in pairs
12	Between two crates	Crate of tubes	Rear right, bottom	Granular pp.	Short bacilli

*NOTES: The tubes with the tested material (*Bacillus subtilis* isolated from hay), were kept in the sterilizer for fifteen minutes at 150° C. They were then removed from the sterilizer, and with extract broth added to them, incubated at 37° C. for twenty-four hours.

the two end ones representing the air temperature of the oven. With the sterilizer in full operation, readings from the three thermometers are taken simultaneously to determine the length of time necessary for the load temperature, as registered by the center thermometer, to rise to 150° C. Naturally, this will vary depending upon the extent and character of the load. When this temperature is reached, actual sterilization is considered to begin and is continued for one and one-half hours. At the end of this period the burners are turned off, the doors being gradually opened to permit the load to cool.

After having established thus the requirements for successful operation of the sterilizer, a series of tests were performed to determine the degree of resistance of bacteria to dry heat. As it was indicated previously, the investi-

gation was centered mainly on the *Bacilli subtilis,* since these are the most frequently met with contaminating agents in the laboratories, being at the same time highly resistant to heat. Observations were also made with staphylococcus taken from stock cultures used in our laboratory. The sources from which *B. subtilis* was isolated were numerous, so as to include as much as possible the usual habitats of the organisms. They consisted of hay, dust, street dirt, and dirt from the animal house of our laboratory, as well as spores

TABLE III*

150° C. THIRTY MINUTES BACILLUS SUBTILIS (HAY) THERMAL DEATH TIME

TUBE	POSITION OF TUBE IN RELATION TO LOAD	CHARACTER OF LOAD IN STERILIZER	POSITION OF LOAD IN STERILIZER	FINDINGS	
				MACROSCOPIC	MICROSCOPIC
1	Tied to crate of Petri dishes	Crate of Petri dishes	Front center, upper left	Pellicle	Long bacilli
2	On top of crate	Crate of tubes	Back bottom, left	Pellicle	Cells in pairs
3	Top of crate. Tube covered with paper	Crate of tubes	Back bottom, right	Cloudy	Long chains
4	In crate. Tube covered with paper	Crate of 4-ounce bottles	Front, center	Granular pp.	Free spores
5	On top of crate	Crate of bottles	Back center	Clear	Negative slide
6	Between crate and wall of sterilizer	Crate of Petri dishes	Front, left	Clear	Negative slide
7	On top of crate. Top of wrapped in paper	Crate of matched plates	Front, upper right	Heavy pellicle	Free spores
8	Under cover of crate	Crate of matched plates	Front, upper right	Pellicle	Short bacilli
9	Top of two crates	Crates of centrifuge tubes	Top, center	Cloudy	Free spores
10	On crate	Crate of tubes	Front, lower left	Pellicle	Short bacilli
11	In crate	Crate of tubes	Front, lower right	Pellicle	Chain formations
12	Between two crates	Crate of tubes	Front, lower right	Pellicle	Chain formations

*NOTES: The tubes were smeared with *Bacillus subtilis* and kept in sterilizer for thirty minutes at 150° C. Extract broth was added and incubated for forty-eight hours at 37° C. After ninety-six hours of incubation one of the clear tubes that showed no growth became cloudy and produced heavy pellicle.

from stock cultures. Besides, efforts were made to approximate in the laboratory as closely as possible the normal environmental conditions of the organisms to produce abundant growths.

Hay infusion was prepared and tubed. The tubes were divided into four sets. Nine tubes of each set were boiled for fifteen minutes, thirty, forty-five minutes, and one hour, respectively. The tubes were subjected to the preliminary boiling for various lengths of time to eliminate the vegetative forms and less resistant bacteria. Following it the tubes were incubated in groups of three of each set at 25°, 30°, and 37° C., respectively, to determine the optimum temperature for their growth and development. When an adequate growth appeared it was transferred to beef extract agar slants and reincubated, thus

stimulating the growth, smears having been taken before and after the addition of the medium, to identify the organisms and the abundance of their spores.

Another source for obtaining highly resistant spores was dust. Sterile test tubes with the cotton plugs removed remained uncovered in the laboratory rooms for twenty-four hours to collect as much dust as possible. Swab sticks were rubbed with dust and put into each tube.

TABLE IV*

150° C. FORTY-FIVE MINUTES BACILLUS SUBTILIS (HAY) THERMAL DEATH TIME

TUBE	POSITION OF TUBE IN RELATION TO LOAD	CHARACTER OF LOAD IN STERILIZER	POSITION OF LOAD IN STERILIZER	FINDINGS	
				MACROSCOPIC	MICROSCOPIC
1	Between bottles. Bottom of tube covered with brown paper	Square crate of bottles	Front, top, center	Pellicle	Free spores
2	Between bottles. Top of tube covered with brown paper	Crate of bottles	Front, upper right	Pellicle	Free spores
3	Between two crates. Entirely wrapped with brown paper	Crates of Petri dishes	Upper front, top shelf	Pellicle	Free spores
4	Between T. B. tubes	Crate of T. B. tubes	Back bottom, center	Clear	Few bacilli
5	Between 2-ounce bottles	Crate of bottles	Front, upper left	Clear	Few bacilli
6	Top of tubes	Crate of potato tubes	Front bottom, center	Clear	No growth
7	Top of 4-ounce bottles	Crate of bottles	Back bottom, left	Pellicle	Spores
8	Attached to crate	Crate of large bottles	Back bottom, left	Clear	No growth
9	Between crate and wall of sterilizer	Crate of potato tubes	Front, upper shelf	Cloudy	Long bacilli
10	Between two crates	Crates of tubes	Back, bottom	Clear	Few bacilli
11	Top of Petri dishes	Crate of Petries	Front, center	Pellicle	Long chains
12	In crate. Wrapped in newspaper	Crate of miscellaneous glassware	Front bottom, right	Cloudy	Chain formation

*NOTES: The tubes with *Bacillus subtilis*, isolated from hay were sterilized for forty-five minutes, at 150° C. Extract broth was added to the tubes. After prolonged incubation tubes with few bacilli produced heavy growths; tubes with no growth did not show any changes.

To obtain suitable spores in addition, dirt was also taken from the street and incubated. The dirt from the animal house containing animal (rabbit and guinea pig) hairs and feces, was put into 10 c.c. of beef extract broth and dilutions made of 1:10; 1:20; 1:40; 1:80; 1:100. These dilutions were incubated at 37° C. for one hour and transferred to beef extract agar pour plates and incubated for twenty-four and forty-eight hours at 37° C. It was noticed, however, that the growth was most abundant in the dilutions of 1:10 and 1:20. In the higher dilutions the number of colonies was considerably decreased. This source was used for the isolation of different types of organisms found in the soil.

The pure cultures of *B. subtilis* isolated from hay were smeared on walls of sterile test tubes under aseptic conditions and these together with organisms

obtained from the other sources as well as staphylococcus cultures were subjected to the same environmental conditions as the glassware placing them in the sterilizer in various positions indicated by the tables and sterilized applying varying temperatures and periods of time.

Besides test tubes, Petri dishes, bottles, and flasks of the sizes and descriptions used in laboratories, wrapped in paper and unwrapped were employed for smearing the bacteria to be tested.

TABLE V*

150° C. ONE HOUR BACILLUS SUBTILIS (HAY) THERMAL DEATH TIME

TUBE	POSITION OF TUBE IN RELATION TO LOAD	CHARACTER OF LOAD IN STERILIZER	POSITION OF LOAD IN STERILIZER	FINDINGS	
				MACROSCOPIC	MICROSCOPIC
1	Under cover of crate	Crates of matched plates	Back, upper right	Clear	No growth
2	Top of crate	Crate of Petries	Back top, center	Clear	No growth
3	Tied to crate. Tube wrapped with newspaper	Crate of matched plates	Back, upper left	Slightly cloudy	Short bacilli
4	Between two crates	Crates of centrifuge tubes	Back bottom, center	Granular pp.	Free spores
5	Tied to side of crate	Crate of Petries	Front top, center	Clear	No growth
6	Bottom of crate. Tube completely covered with brown paper	Crate of test tubes	Back bottom, left	Cloudy	Long bacilli
7	Center of crate	Crate of potato tubes	Back bottom, right	Clear	No growth
8	Tied to crate. Top of tube covered with brown paper	Crate of Petries	Back bottom, center	Slightly cloudy	Few bacilli
9	In a rack	Rack for Smith fermentation tubes	Front bottom, right	Clear	No growth
10	Tied to metal container	Metal containers of pipettes	Front bottom, left	Clear	No growth
11	Center of crate. Top of tube covered with brown paper	Crate of flasks	Front bottom, center	Pellicle	Spores
12	Between crate and wall of sterilizer	Crate of bottles	Bottom, right	Clear	No growth

*NOTES: Tubes smeared with *Bacillus subtilis* isolated from hay, were kept in sterilizer at 150° C. for one hour. Extract Broth added and incubated at 37° C. for various periods. No changes occurred after one month of incubation.

Bacillus subtilis isolated from hay was carried to twenty generations in order to gradually attenuate their resistance. The material to be tested was exposed to 140° C. for one and one-half hours; to 150° C. for fifteen, thirty, forty-five minutes, one hour, and one and a half hours; 160° C. for fifteen minutes and one hour. After each sterilization the test tubes, Petri dishes, bottles, and flasks with the planted organisms were removed from the sterilizer; beef extract broth and beef extract agar were added to them and cul-

tures incubated at twenty-four, forty-eight, seventy-two, ninety-six, etc. hours at 37° C. and tested for the presence of actively living or dormant organisms.

The organisms found in the samples of dirt from the animal house and the *B. Subtilis* from hay were most resistant to heat. However, all were destroyed at 150° C. for one and a half hours and at 160° C. for one hour.

At 150° for fifteen, thirty, and forty-five minutes most of the spores survived, some though at the longer periods were dormant. Those which were exposed at 140° C. were not killed after one and one-half hours of sterilization.

TABLE VI*

150° C. ONE AND ONE-HALF HOURS VARIOUS ORGANISMS THERMAL DEATH TIME

TYPE OF GLASSWARE USED IN TEST	MATERIAL SEEDED	POSITION OF GLASSWARE IN RELATION TO LOAD	CHARACTER OF LOAD	POSITION OF LOAD IN STERILIZER	FINDINGS
Petri dish	Subtilis X	Top of crate	Crate of potato tubes	Front bottom, center	No growth
Petri dish	Dirt A	Bottom of crate	Crate of Petries	Front top, right	No growth
Petri dish	Subtilis S	Between crate and wall of sterilizer	Crate of matched plates	Front top, left	No growth
Petri dish	Dirt B	Center of crate	Crate of tubes	Back bottom, left	No growth
8-ounce bottle	Staphylococcus	Attached to crate. Bottle entirely wrapped with paper	Crate of Petries	Center, top shelf	No growth
Test tube	Dust	Bottom of crate	Crate of bottles	Back top, right	No growth
4-ounce bottle	Dirt A	In crate	Crate of tubes	Front, top shelf	No growth
Potato tube	Subtilis S	Bottom of crate	Crate of matched plates	Front, bottom	No growth
Potato tube	Dirt B	Attached to crate. Tube wrapped with brown paper	Crate of bottles	Center, bottom	No growth
Petri dish	Subtilis X	Between pipettes	Packages of pipettes	Front top, left	No growth
Test tube	Dust	In crate	Crate of matched plates	Back bottom, left	No growth
300 c.c. flask	Staphylococcus	In crate	Crate of miscellaneous glassware	Front top shelf, right	No growth

*NOTES: Subtilis X, pure culture isolated from hay; Subtilis S, stock culture used in our laboratory; Dirt A, from our animal house; Dirt B, from the street; Dust, collected in the laboratory; Staphylococcus, stock culture.

These various tubes, bottles, flasks, and Petri dishes with the material to be tested were sterilized at 150° C. for one and one-half hours, extract broth and extract agar added and cultures incubated at 37° C. for ninety-six hours and longer. No growth appeared.

SUMMARY

In order to insure absolute sterility, regardless of whether the organisms are more or less heat resistant, the sterilization periods should not be less than one and one-half hours and the temperature should be between 155° to 160° C.

Acknowledgement.—The author wishes to acknowledge her gratitude to: Dr. William Hallock Park for his guidance and valuable suggestions, Mr. Charles Tyler for the checking of the temperatures with thermocouples, Mr. Shuler of Bramhall Deane Co., of New York, and Miss Henrietta Gartman great appreciation for assisting with the technical part of the work.

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A COMPARATIVE STUDY OF DEHYDRATION*

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THE acetone method is known to be a short and reliable way of dehydrating tissue. Tertiary butyl alcohol has been used by Johansen (1935) with considerable success as a substitute for ethyl alcohol. He believes that a great deal of the shrinkage which has been blamed on fixation was really caused by ethyl alcohol and the commonly used clearing agents. He is of the opinion "that no blame can be placed upon the fixing fluid if disaster results during postfixation stages. In other words, 'successful' dehydration and infiltration is not dependent upon 'successful' fixation: the two processes are mutually exclusive."

Dioxane (diethylene oxide) has been used with varying results by several investigators among the first of whom were Graupner and Weissberger (1931) who describe the use of dioxane in dehydrating tissue before sectioning. They transferred the tissue from the fixative through dioxane containing calcium chloride to a mixture of paraffin and dioxane into pure paraffin. Very good results were obtained. Johansen (personal communication) obtained very inconsistent results with the use of dioxane.

Cellosolve (ethylene glycol mono-ethyl ether), a commercial product used in the cellulose lacquer industry, was recently introduced, as a dehydrating agent, by Frost (1935). Frost was able to dehydrate, stain and mount plant tissue in five minutes.

Other reagents have been suggested as substitutes for ethyl alcohol. Some of these have been used with some success in botanical work but none have come to be widely used in the preparation of animal tissues. In most of the techniques the substitute reagents have not entirely replaced ethyl alcohol and some have even been mixed with ethyl alcohol. Among these substitutes for ethyl alcohol may be mentioned a mixture of ethyl alcohol and n-butyl alcohol by Larbaud (1921) and Zirkle (1930), n-propyl alcohol by Sheridan (1929) and iso-propyl alcohol by Bradbury (1931). Baird (1936) made a comparative study of dioxane, iso-butyl alcohol and ethyl alcohol with chloroform on several animal tissues. These were used in dehydration before embedding and were not employed in the staining process. She obtained the best results with dioxane. Mossman (1934) as well as Bucher and Blakely (1936) discuss the use of dioxane in place of ethyl alcohol and the common clearing agents in the paraffin technique. The latter investigators report that preparations were completed in 48 hours. The method described in the work here reported requires a great deal less time.

METHODS

A comparative study of the dehydrating qualities of 1, 4 Dioxane, cellosolve, tertiary butyl alcohol and acetone have been made on some normal and abnormal human tissues following several fixatives. In all cases control slides were made

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of the same tissue using the familiar ethyl alcohol technique followed by a common clearing agent. The human tissues used were pancreas, kidney, liver, cardiac muscle, cervix uteri, cancer of the breast, cancer of the uterus, and tumor of the neck. Some tissues were removed at autopsy and others at biopsy. It should be pointed out that in the technique described here more emphasis has been placed on securing histologic preparations sufficient for diagnosis rather than for cytologic details. Work is in progress on the latter aspect of the problem.

Fixation.—Among the various fixatives used were Bouin's, Orth's, 10 per cent formalin, 20 per cent formalin and hot 100 per cent formalin. Bouin's fluid was perhaps the least successful due to the fact that the tissue must be washed in diluted ethyl alcohol or one of the dehydrating agents for a con-

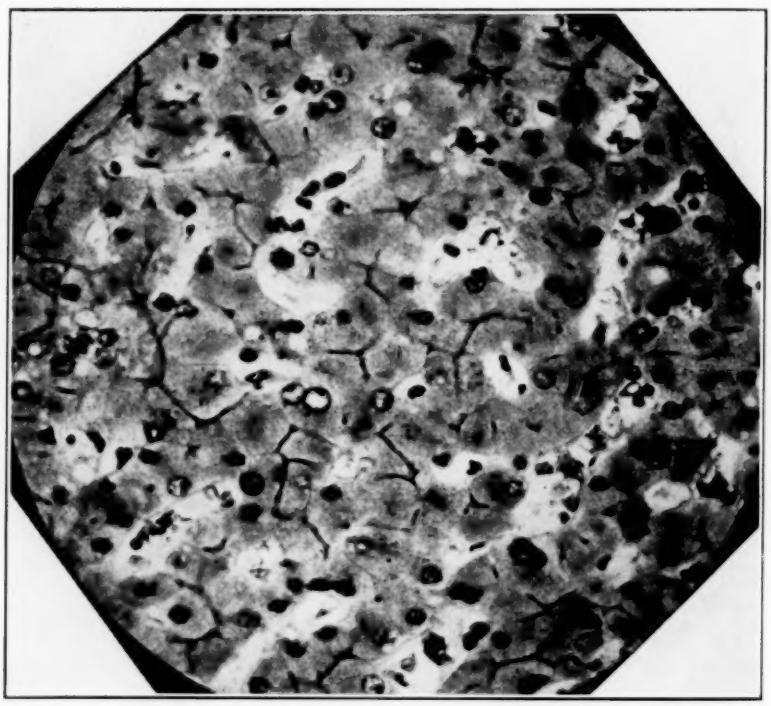

Fig. 1.—Human liver. Fixed in hot 100 per cent formalin and dehydrated in acetone. Iron hematoxylin.

siderable time, to remove the pieric acid, for good staining results. By far the most successful fixative used, from the standpoint of speed, was full strength commercial formalin heated almost to the boiling point, after which the tissue was washed in running water for a few minutes to remove the excess formalin.

Dehydration.—Tissue was transferred directly from the fixative or water, as the case may be, to the undiluted dehydrating agent. It is very important that the dehydrating fluid be perfectly "dry." In the case of acetone and tertiary butyl alcohol chemically pure products were used and they no doubt contained a minimum of water; however, the addition of a small piece of tissue adds enough water to spoil them for further use. For keeping the reagents "dry" generous amounts of anhydrous copper sulphate and calcium chloride

were tried. It was found that copper sulphate was best in all the fluids as it would not only take up more water than calcium chloride but one could tell by the color change when the fluid was too "wet" to use. Calcium chloride was fairly successful with dioxane and acetone but not with tertiary butyl alcohol and cellosolve. The tissue may be suspended in the fluid in cloth bags or placed directly on the layer of copper sulphate.

For complete dehydration from one to one and one-half hours is required, depending on the size and texture of the tissue. Two changes were usually sufficient, that is, the tissue was placed in the first container for one-half hour and in the second for about an hour.

Although all of the reagents are more expensive per unit volume than ethyl alcohol they can be used repeatedly as long as they are kept "dry." When this is considered it is found that dioxane and acetone, for example, which can be used longer than tertiary butyl alcohol and cellosolve, are as cheap as or cheaper than ethyl alcohol.

Fig. 2.—Human liver showing structures in relief. Fixed in hot 100 per cent formalin and dehydrated in acetone. Iron hematoxylin.

Embedding.—Tissue was transferred directly from the dehydrating agent to pure melted paraffin with no intermediate steps. In the case of tissue transferred from acetone to melted paraffin, bubbles form on the surface of the tissue and may cause it to rise to the surface. This reaction lasts only a short time.

Infiltration with paraffin is as rapid following acetone and dioxane as after chloroform, toluene or xylene. In no instance was infiltration imperfect following these two reagents when they were kept "dry." Infiltration was most difficult after cellosolve. This was perhaps due to water and other impurities since cellosolve is a commercial product and not chemically pure. It is probable that a purer fraction would prove to be more successful in this type of work.

No difficulty was encountered in sectioning after any of the methods, provided, of course, that infiltration was complete. It was thought that sectioning was easiest following dioxane and cellosolve. Acetone often caused a certain amount of brittleness, especially in malignant tissues.

The sections were mounted on the slides with generous amounts of Mayer's albumin after which they were allowed to dry thoroughly. If they are not dry they may come off during the staining process.

Staining.—After the sections are completely dry the slide is placed in xylol until the paraffin is removed. It is not known how much, if any, damage occurs to the tissue while in xylol during this short time. Xylol is the best paraffin solvent that has been tried though dioxane is a fairly good solvent if used hot; but it is not as satisfactory as xylol. Next the slide is transferred to the undiluted dehydrating agent for a minute or more, then into distilled water until the dehydrating fluid is removed. Here, as in all methods, the time for each step may be shortened if the slide is gently moved around to accelerate the exchange between tissue and fluid. Next the slide is stained with Delafield's hematoxylin from which it is transferred to slightly alkaline tap water until sections are blue. Usually no destaining is necessary, but if desired destaining may be

Fig. 3.—Human liver. Fixed in 20 per cent formalin and dehydrated in acetone. Iron hematoxylin. Varicosities of bile capillaries can be seen.

accomplished in acidified tap water. After tap water it is advisable, but not necessary, to pass the slide through distilled water. At this point the procedure varies slightly for the different reagents used. Aqueous eosin may easily be dissolved in acetone and cellosolve until the desired strength is reached. There, when double staining, both staining and dehydration may be accomplished in the same step. However, it is difficult to dissolve enough eosin in tertiary butyl alcohol and impossible to dissolve any in dioxane for staining purposes. So in the case of the latter two reagents it is necessary to use ordinary aqueous eosin. In all cases it is desirable to wipe the excess water from the slides before placing in the dehydrating agent as this enables them to be used for a longer time. A total of two changes in each of the dehydrating agents is best although one is

often enough. Mounting is done with Canada balsam direct from the dehydrating fluid except in the case of acetone which must be passed through xylol. Acetone is not miscible with balsam and the evaporation is so rapid that the sections dry before mounting is possible. By using the above technique good preparations were made in three to five minutes.

Observations.—A piece of tissue was cut into cubes measuring approximately one-half centimeter and each block was treated identically except that each was treated with a different dehydrating agent. Also in all cases a similar block was treated with the usual ethyl alcohol technique. It should be understood that with the exception of the ethyl alcohol no graded series were used.

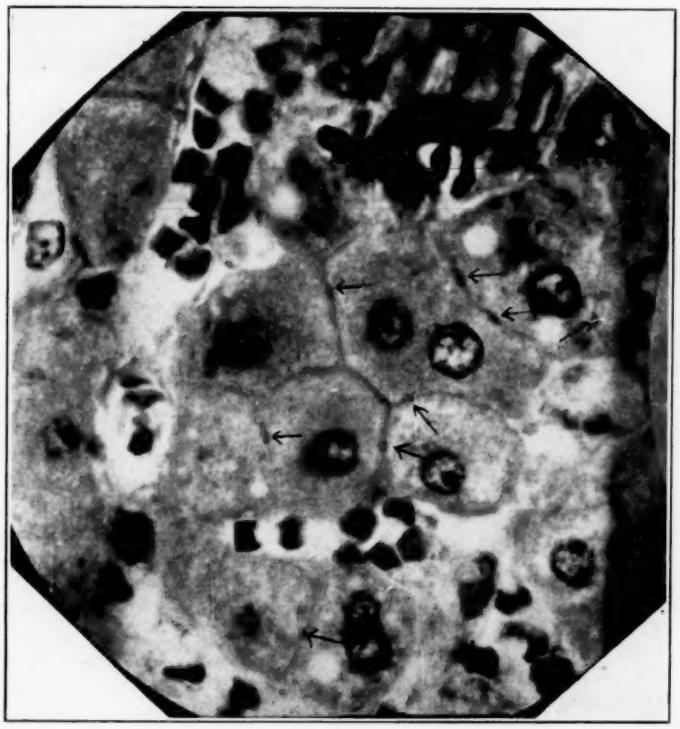

Fig. 4.—Human liver. Fixed in Orth's fluid and dehydrated in dioxane. Iron hematoxylin. Bile capillaries showing no varicosities are present. Arrows point to cut ends of bile capillaries.

When the completed slides were compared some differences were apparent. In every case the best slides were obtained by using dioxane or acetone. They were not only better than those which had been dehydrated with cellosolve and tertiary butyl alcohol but were better than those which had been carefully dehydrated with ethyl alcohol and cleared in chloroform. And of course such a technique required only a fraction of the time needed for ethyl alcohol dehydration.

The preparations made using tertiary butyl alcohol and cellosolve were, in most cases, very poor, the cellosolve giving somewhat the better results. However, cellosolve seemed to cause more shrinkage, especially nuclear shrinkage, than any of the other reagents. On the other hand it was most difficult to stain

sections following tertiary butyl alcohol. This was especially true with cancerous tissue, which took a very uneven stain following this fluid. Tertiary butyl alcohol caused a great deal of shrinkage in connective tissue while acetone seemed to cause the least. In fact, the question has arisen, concerning acetone, as to whether it does not even cause a slight swelling of the collagen fibers.

In all of the preparations the cells were more granular than after the use of ethyl alcohol, a result due perhaps to fewer of the cell constituents being dissolved out by these fluids. Because of this the sections often appear more opaque than when ethyl alcohol is used, which may be a disadvantage in some cases. The best preparations of liver were obtained with the use of dioxane following fixation in hot 100 per cent formalin and Orth's fluid. The bile capillaries appeared very distinct and were probably more normal than after acetone treatment where there was some distortion as evidenced by slight contractions and dilations along the capillary.

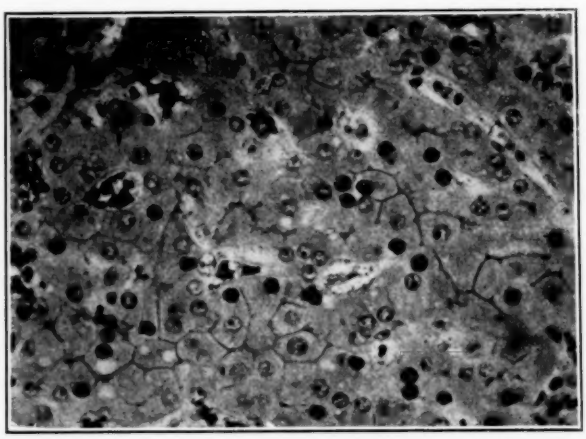

Fig. 5.—Human liver. Fixed in hot 100 per cent formalin and dehydrated in dioxane. Iron hematoxylin. Shows many bile capillaries.

None of the preparations showed anything unusual when examined with polarized light. The striations in the cardiac muscle appeared equally clear following all dehydrating fluids.

Frozen sections were made of some of the tissues and each of the dehydrating fluids was tried on the sections in place of ethyl alcohol. In this material it was found that the best results were obtained with dioxane and tertiary butyl alcohol and that acetone gave the poorest results. This is mentioned, not because any are superior to the ethyl alcohol technique in frozen material but merely to point out that certain of these reagents may be used as a substitute for ethyl alcohol.

It should not be inferred that tertiary butyl alcohol and cellosolve are discredited as dehydrating agents if used differently, perhaps in a graded series. Cellosolve has been used successfully by Frost (1935) on plant tissue. Tertiary butyl alcohol was introduced as a dehydrating agent and used successfully on a variety of materials by Johansen (1935). However, I gather from Johansen's paper that he used a graded series, was interested more in cellular details and was not concerned with the time element.

WARNING

It has recently been reported that the breathing of even a small amount of dioxane fumes causes bronchial, liver and kidney disturbances of a very serious nature. As a result it has been suggested that all work with dioxane be done in a chemical hood. Its use has been forbidden in some British laboratories because of its danger. However, where no hood is available and where the work takes but little time it should be sufficient to work in a well ventilated room and keep the dioxane containers covered at all times when not in use.

SUMMARY

1. A comparative study of four dehydrating agents was made on human tissue following various fixatives. The reagents used were cellosolve, tertiary butyl alcohol, dioxane and acetone. Best results were obtained with the latter two.

2. Briefly summarized into steps the technique is as follows: (1) Fix in hot 100 per cent formalin for five minutes. (2) Wash in running water ten minutes. (3) Dehydrate in two changes of undiluted dehydrating agent for one to one and a half hours. (4) Embed in paraffin. (5) Section. (6) Remove paraffin in xylol. (7) Undiluted dehydrating agent. (8) Distilled water. (9) Stain in hematoxylin. (10) Alkaline tap water until blue. (11) Distilled water. (12) Stain in eosin dissolved in dehydrating agent. (13) One change of dehydrating agent. (14) Mount in Canada balsam. Total time needed from unfixed tissue to completed preparation is about two hours.

It is with pleasure that I acknowledge the following, without whose help this work could not have been accomplished. Zoological Laboratory, University of Cincinnati; Charles Goossmann, M.D., Cincinnati Radium Laboratory; and Cincinnati Deaconess Hospital.

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APPARATUS FOR THE EXTRACTION OF ESTRIN FROM URINE*

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THE apparatus† described below was designed for the isolation of estrin from urine. The process is continuous and requires no attention during the period of extraction. The apparatus is inexpensive, rapidly assembled, and sufficiently lacking in rigidity to avoid being fragile. In principle, droplets of chloroform are passed through acidified urine, and the resultant extract flows by gravity into a flask from which the chloroform is evaporated to leave behind the hormone. The vaporized chloroform is subsequently condensed, and passage through the urine repeated.

I. DESCRIPTION OF APPARATUS

Dimensions of the several parts of the apparatus are shown in Fig. 1. All glassware is Pyrex. Flask A is a standard product (ground joint No. 20) into which a safety tube has been inserted. The latter reaches almost to the bottom of the flask and is flared at the outer end. Relatively wide bore glass tubing is employed (11 and 10 mm.), in order to make the apparatus as sturdy as possible. The Liebig type condenser of the size shown, is adequate in effecting condensation of chloroform vapor. Flask B is a stock separatory funnel modified by the attachment of the delivery tube, as shown. The coil in B consists of glass, 1 cm. wide, wound tightly about a glass rod, and supported at the bottom by three prongs. The diameter of the opening in the stopcock shown is 4 mm.

Fig. 2 is a photograph of the assembled apparatus. Flask A rests on an asbestos centered wire gauze. The source of heat is a General Electric hotpoint plate (diameter 6 inches). The 11 mm. tube is steadied with a buret clamp, and connected with the condenser by means of gum tubing. The joint is glass to glass. If the latter precaution is observed, there is no need for a ground glass connection at this point. A contort clamp holds the iron rod which supports the condenser. Flask B rests within an iron ring (inside diameter 4¼ inches), and the coil inside is arranged to avoid touching the neck of the vessel. The 10 mm. tube delivers into the flared portion of the safety tube (Flask A). No rubber stoppers or corks are necessary.

II. PROCEDURE

From a twenty-four-hour sample of urine previously adjusted to pH 1.5 (conc. H_2SO_4), 500 c.c. are withdrawn for extraction. The urine is placed in Flask B (stopcock closed), 250 c.c. chloroform added, and the glass coil inserted. In Flask A 500 c.c. chloroform are placed. The apparatus is now assembled as shown in Fig. 2. The condenser is connected with a source of cold water, and

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†Apparatus made by Eck and Krebs, New York, N. Y.

heating begun at *medium*. When choloroform has begun to boil, the plate is turned down to *low*. Delivery of chloroform from the condenser should consist of a continuous series of drops falling upon the central portion of the glass coil.

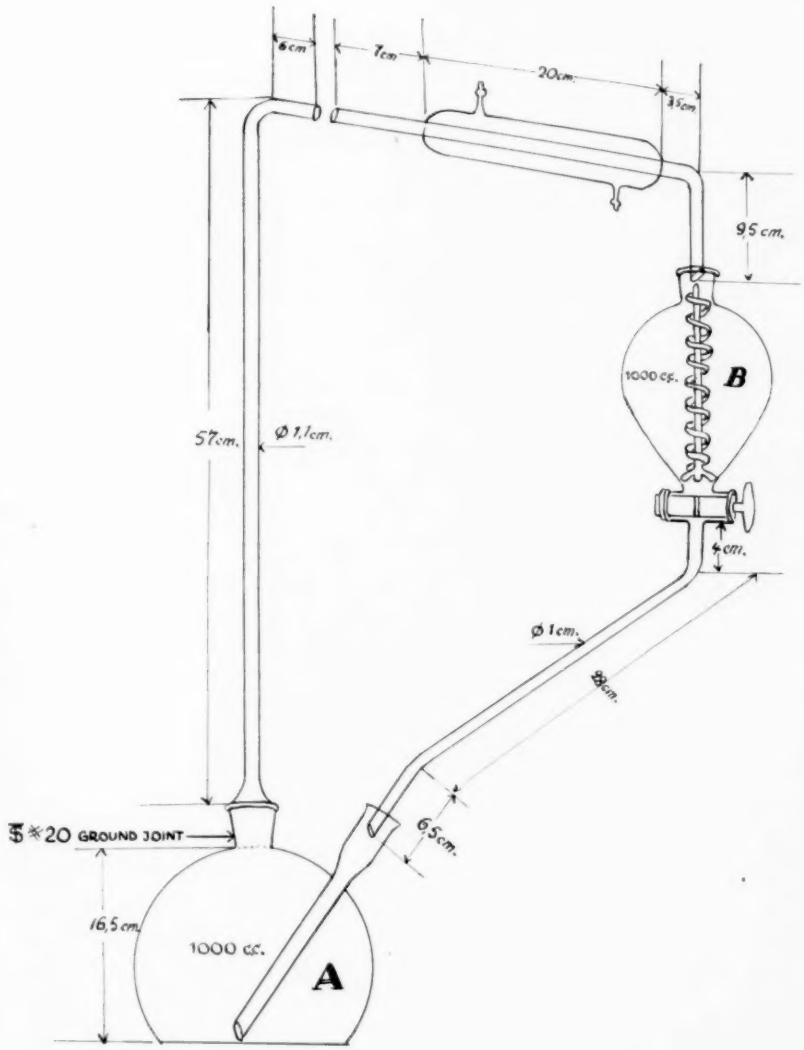

Fig. 1.

The object of the latter is to retard the passage of chloroform through the urine, in order to make the extraction process more efficient. Shortly after distillation has begun, the stopcock on B is opened sufficiently to counterbalance the inflow of chloroform. Extraction is continued for six hours. If not subjected to pro-

longed changes of current, or to a lowering of temperature caused by drafts, the apparatus will continue to operate without need of adjustment during the entire period of extraction.

When extraction is completed, distillation is discontinued, and chloroform in B drained into Flask A. Flask B is removed, the urine discarded, and chloroform in A distilled into a clean, dry container. Recovery of chloroform can be

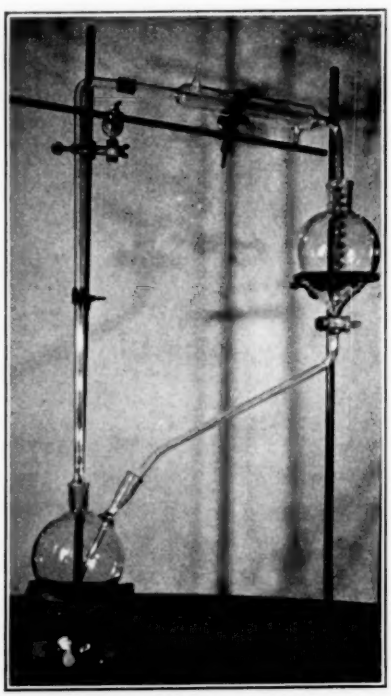

Fig. 2.

carried out with the plate either at *medium* or *high*. The final 100 c.c., however, are boiled off at *low*. Heating is discontinued just before the level of liquid in the flask has sunk below the mouth of the safety tube. The flask is then removed from the plate, cooled under running water, and the contents poured into a dry 50 c.c. beaker. The flask is washed with two 5 c.c. portions of chloroform, and the washings added to the hormone extract. Combined washings and extract may be further concentrated on a water-bath to any desired volume. After the addition of olive oil, the estrin is ready for animal testing.

A NEW CHOCOLATE AGAR FOR CULTURE OF THE GONOCOCCUS*

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SINCE the discovery of the gonococcus by Neisser, numerous media have been devised and reported for its isolation and culture. Outstanding among these have been the hormone media of Huntoon,[1] Spray's modification[2] of North's[3] medium, and the chocolate agar of Spohr and Landy.[4] The latter make use of North's gelatin agar (Difco), enriched with sheep blood, which is added with the agar at 75° C., and is prepared just immediately preceding use in order to insure a moist surface.

In a study being conducted on the isolation and cultivation of the gonococcus, all of the usual media proved unsatisfactory for one reason or another. Due to the limited time available and the large amount of material being examined, it was found necessary and desirable to prepare the media in fairly large quantities, sufficient to last several weeks. Considerable difficulty was likewise experienced in getting positive cultures from patients who had only a slight infection or in those who had few gonococci of a weakly growing strain present in the discharge. In the majority of these cases the contaminating organisms rapidly overgrew the gonococci before isolation or identification could be effected.

A medium was sought which would enhance the growth of these weakly growing or slow developing strains, allowing a more rapid isolation and identification in pure culture.

The medium finally selected as most nearly meeting the requirements was prepared as follows:

> Take 77 grams of Bacto-North Gelatin Agar (Spray's formula) and dissolve in 1,000 c.c. of distilled water by boiling several minutes. The medium thus prepared is dispensed in 1,000 c.c. Erlenmeyer flasks, 500 c.c. in each flask. The flasks are stoppered with gauze and cotton plugs, capped with heavy brown Kraft paper and autoclaved at 20 pounds for thirty to fifty minutes. Flasks are cooled to 55° to 60° C., 50 c.c. of citrated beef blood added to each, and gently rotated to insure thorough mixing of blood and agar. At this temperature an even distribution of blood throughout the agar is easily obtained, whereas at higher temperatures the blood tends to coagulate before it can be thoroughly distributed, causing lumps in the final medium. The flasks are then placed in a water bath at approximately 60° to 65° C., and the temperature gradually raised to 100° C. About fifteen to twenty minutes should be consumed in raising to this temperature. The blood and agar mixture must be frequently agitated by gently rotating the flask, during the entire heating process, care being exercised to prevent foaming. Hold at 100° C. for twenty minutes. At the end of the heating period, the

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agar is rapidly cooled to about 55° C. and poured into petri dishes, 15 to 20 c.c. each. Cooling below this temperature makes the agar extremely difficult to pour, as it congeals at about 45° C.

The advantages of this medium over others used are numerous, outstanding among which are:

1. Beef blood is used, which can be obtained at any slaughter house. It need not necessarily be collected aseptically, only the usual precautions of cleanliness being observed. The heating process is sufficient to kill all ordinary contaminants. All plates are given a preliminary incubation before use, and no contaminants have been observed on any plates before inoculation.

2. Petri dishes may be prepared well in advance of the time they are to be used. Plates prepared and kept at ice box temperature four to five weeks were found to give results equally as satisfactory as freshly prepared ones.

3. Excellent growth of even the more weakly growing strains is obtained in twenty-four hours, and in many cases, colonies of the gonococcus have been observed before any of the contaminants were visible.

4. A higher percentage of positive results was made possible on borderline cases with this medium.

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A NOTE ON THE STAINING OF TUBERCLE BACILLI IN SECTIONS*

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IT IS commonly believed that fixation of tissues in formalin renders them unsuitable for staining acid-fast bacteria. A method for staining tubercle bacilli which gives satisfactory results after formalin and after Zenker-formol seems, therefore, worth recording. The method which follows yields good staining of the bacteria with either of these fixatives. With formalin-fixed tissue the green counterstain is prone to fade in a few months, the bacteria and nuclei remaining well stained indefinitely. With tissue fixed in Zenker-formol, all structures retain the dyes for prolonged periods.

In general the method consists of staining in iron hematoxylin, followed by fuchsin, and employing light green as a counterstain. This gives a clear staining of the nuclei; the nuclear membrane is dark blue green and the nucleoli are blue green, with a paler light green for the cytoplasm against which the red bacilli show up brilliantly.

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PROCEDURE

1. Run slides down to water as usual and rinse in distilled water.
2. Mordant in iron alum solution in the oven for five minutes (45° to 50° C.).
3. Rinse at the tap.
4. Stain in hematoxylin, also in the oven, for five minutes.
5. Put directly into the picric acid for five minutes, or longer if necessary, until the stain remains only in the nuclei.
6. Wash thoroughly in running water (fifteen minutes or longer) until the sections are thoroughly free of the picric acid.
7. Place on the heating bar and pour the fuchsin solution over them. Heat until steam comes off, but do not boil. Let cool for three minutes, heat again, and again allow to cool for three minutes.
8. Destain in acid alcohol for a few seconds, until the fuchsin starts coming off in clouds; rinse in tap water and continue destaining until the sections are barely pink.
9. Rinse in water and put into alkaline water for a few seconds.
10. Wash thoroughly in running water for ten minutes or more.
11. Stain in the light green solution for five minutes.
12. Rinse in water and run up quickly through the alcohols and xylol; mount as usual.

SOLUTIONS

1. *Iron alum.*—5 per cent aqueous solution of iron alum.
2. *Hematoxylin.*—Dissolve 1 gm. of Grübler's hematoxylin in 80 c.c. of hot distilled water, cool, and add 10 c.c. of glycerin and 10 c.c. of 95 per cent alcohol.
3. *Picric acid.*—Add 2 parts of a saturated alcoholic (95 per cent) solution of picric acid to 1 part of 95 per cent alcohol.
4. *Fuchsin.*—Add 16 c.c. of a saturated alcoholic (95 per cent) solution of fuchsin to 84 c.c. of aniline water.
5. *Acid alcohol.*—3 per cent nitric acid in 95 per cent alcohol.
6. *Alkaline water.*—Add a little ammonia to distilled water.
7. *Light green.*—1 per cent aqueous solution of light green (Grübler).

DISCUSSION

This method has been used over a period of three years and has been applied to tuberculous tissues from several animal species inoculated with various strains of tubercle bacilli. Aside from technical facility, it has certain other distinct advantages. It gives good nuclear and cytoplasmic staining, so that fine cellular differentiation is obtained. This is particularly evident in such tissues as bone marrow, in which sections so stained are useful not only for studies of tubercle bacilli but also for studies of the marrow cells.

The method is excellent for purposes of microphotography, as tubercle bacilli are stained brilliantly red while nuclei and cytoplasm take different values of green so that excellent contrast is obtainable. For ordinary microscopic examinations, the red-green contrast has been found superior to other staining methods.

By this method tubercle bacilli are equally well stained in sections from all viscera. Whereas certain other methods have been found unsatisfactory for staining these organisms in brain and meninges, the technique described herein has been found highly satisfactory in a large series of animals subjected to intracerebral inoculation. Intracellular organisms, so often appearing purple or black when stained by other methods, are brilliant red in this method and are, therefore, easily distinguishable from nonacid-fast bacteria or nonbacillary material. There has been no evidence that bacteria other than tubercle bacilli retain the fuchsin; moreover, distinctive characteristics of individual bacteria, such as beading, are well maintained.

THE GRAPHIC REPRESENTATION OF THE BLOOD PICTURE

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SINCE the significance of the leucocyte count depends upon a number of factors, the ideal way of expressing it would be in the form of a curve, provided the number of factors to be expressed were not too great. Such a curve would be valuable in showing at a glance the significance of the leucocyte count, and in that it would enable the blood picture during the course of an illness to be charted. By means of the method herein described, a curve may be constructed, which will express the severity of the infection, the degree of resistance, and the number of immature neutrophiles, both in a qualitative and quantitative manner. It is based upon the Gibson Chart, but expresses, in addition, the number of immature neutrophiles, both qualitatively and quantitatively. In our opinion, any chart or graph of leucocytes which fails to include this factor is inadequate.

While in the early stages of an acute pyogenic process, there may be no significant changes in the number of immature neutrophiles, there are times when it is the chief, if not the sole factor of any value, in determining the prognosis. Nicholson states that the immature-mature count taken at four- to eight-hour intervals is a better guide to the patient's condition than the temperature chart.

METHOD

The curve to be described is not dissimilar to that which has been devised to express the significance of the blood sedimentation rate. As in the case of the sedimentation curve, a curve of one degree of slope means one thing, a curve of less slope, another, and a straight line bears still another significance. The principle of the Gibson Chart has been taken as the basis for constructing the curves. A horizontal curve (i.e., a curve whose ends are on a horizontal line) indicates that the resistance equals the infection. Curves sloping downward to the right indicate in a general way that the infection is less than the resistance; while curves sloping upward to the right suggest that the infection is in excess

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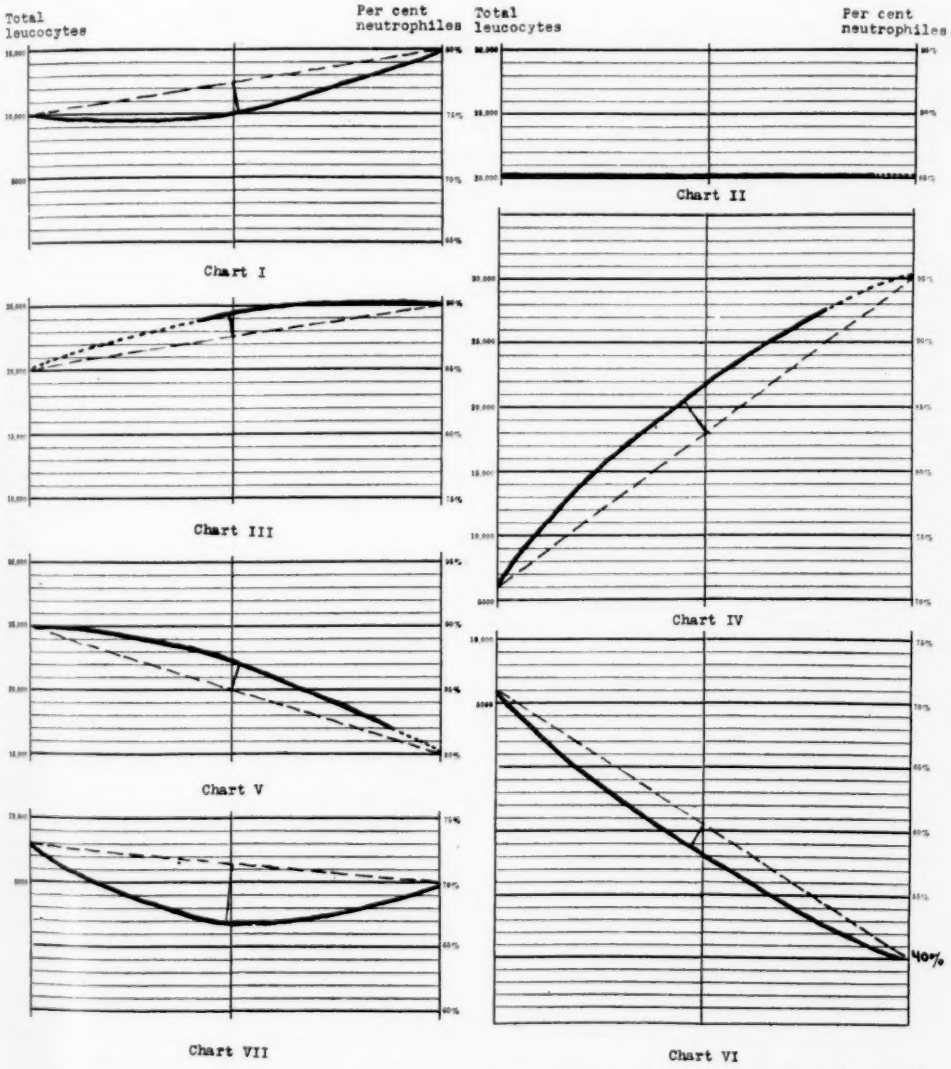
of the resistance. According to Neal, if the neutrophile percentage is above the white count to the extent of five divisions in surgical cases, it is an indication for operative intervention.

To express the degree of increase in immature neutrophiles, an arbitrary scale has been chosen corresponding to the divisions on the chart. The percentage of immature neutrophiles is given by the distance of the midpoint on a



Charts I to VII.—The curve of the blood picture in various diseases. I.—A typical severe bronchitis: total leucocytes 10,000; per cent neutrophiles 80; staff cells 35; segmented neutrophiles 45. II.—Severe pneumonia: total leucocytes 20,000; per cent neutrophiles 85; staff cells 40; metamyelocytes 10; segmented neutrophiles 35. III.—Fatal pneumonia: total leucocytes 20,000; per cent neutrophiles 90; staff cells 45; metamyelocytes 15; myelocytes 5; segmented neutrophiles 25. IV.—Fatal pneumonia, with leucopenia: total leucocytes 6,000; per cent neutrophiles 95; staff cells 50; metamyelocytes 20; myelocytes 10; segmented neutrophiles 15. V.—Typhus fever: total leucocytes 25,000; per cent neutrophiles 80; staff cells 60; metamyelocytes 10; myelocytes 1; segmented neutrophiles 8. VI.—Malta fever: total leucocytes 6,000; per cent neutrophiles 40; staff cells 30; segmented neutrophiles 10. VII.—Normal leucocyte count: total leucocytes 8,000; per cent neutrophiles 70; staff cells 5; segmented neutrophiles 65. (Dotted portion of curve represents red; solid portion represents blue.)

curve, drawn from the figure for the total number of leucocytes on the left to the neutrophile percentage on the right, from a dotted line joining the same points. Each division on the chart represents an increase of 10 per cent in the number of immature neutrophiles. Myelocytes, metamyelocytes, and staff cells are included in the immature neutrophiles in determining the distance of the curve from the dotted line. All curves above the dotted line represent percentages above fifty, and curves below the dotted line represent percentages below fifty. A curve with a distance from its midpoint to the dotted line of five divisions, below the dotted line, represents a zero percentage of immature neutrophiles. A distance of two and one-half divisions represents a percentage of twenty-five, etc. When the curve and the dotted line coincide, the immature percentage is equal to fifty. According to Osgood, a staff count above 50 per cent usually indicates a fatal outcome; therefore, all curves above the dotted line may be said to suggest a fatal outcome.

In order to express the increase in immature neutrophiles qualitatively as well as quantitatively, it is necessary to construct the curve of two different colors. Thus, a count composed of 25 per cent juveniles (metamyelocytes) may be represented by drawing one-fourth of the curve in red, and the remainder in blue. In this way, one can tell at a glance whether the shift is composed entirely of staff cells (degenerative) or if it contains juveniles as well (regenerative), and, in addition, can perceive the relative amounts. Also, when a number of charts are made throughout the course of an illness, the point at which juveniles and other less mature forms first begin to appear, or "the first danger signal" in some cases of infection, can be detected.

To construct the curves with the least effort, a square shaped like a "T" should be used. The vertical line in the center of the chart gives the midpoint of any line drawn across the chart. By placing the base of the "T" across the chart, the dotted line connecting the figure for the white count with the neutrophile percentage can be drawn. Then the perpendicular can be drawn from the center of the dotted line along the leg of the "T," which has been marked off in divisions corresponding to those on the chart. The curve is then drawn by connecting the two ends with the midpoint, located at the proper distance from the dotted line along the leg of the "T." To construct the curve in colors, a red and blue pencil may be used, such as may be purchased at any printer's supply house.

DISCUSSION

When this method has been applied in a number of infectious diseases and pathologic states, it is seen that several different curves with rather characteristic forms result, enabling the hematologist at once to place the curve in one of the various classes. There is a conspicious difference between the "infectious fever type" of curve with good resistance, and a moderate increase in immature neutrophiles (as in Malta fever), and the "toxic type" (as in pneumonia), where the increase in immature neutrophiles is very marked, and the infection is greatly out of proportion to the patient's resistance. Various other forms of the curve are seen in appendicitis and other acute abdominal conditions. In infections of a more or less minor nature, the curve approaches the normal type.

In our consideration of this subject, we at first thought of using the immature-mature ratio, or "filament-nonfilament count," as it is sometimes called, as the basis for constructing the curve of the immature forms. This is still taught in some medical schools and in some textbooks as the method for determining the prognosis from the "shift to the left." However, it was found that the immature-mature ratio varies to such an extent with changes in the percentage of total neutrophiles, that its value in prognosis is questionable. For instance, it is possible for the immature forms to be considerably in excess of the mature, while the patient is in no way dangerously ill. This naturally follows, since as the number of total neutrophiles decreases, the smaller the degree of increase in the immature forms necessary to make a marked change in the immature-mature ratio. *The physician, therefore, should not follow the rule that is occasionally used, that when the immature forms have increased to the point where they are equal to, or in excess of the mature forms, the prognosis should be considered grave.* If one wishes to use the immature-mature ratio in prognosis, it is necessary to use the rule given by Osgood, that 50 per cent staff cells suggest a fatal outcome, and to determine the significance of the immature-mature ratio at each change in the neutrophile percentage. This may be tabulated somewhat as follows:

PERCENTAGE OF TOTAL NEUTROPHILES	MINIMUM IMMATURE-MATURE RATIO INDICATING A FATAL OUTCOME
100%	1:1
95%	1.11:1
90%	1.25:1
80%	1.66:1
70%	2.50:1
60%	5.00:1
55%	10.00:1
50%	100% Immature

It would appear, therefore, that below 50 per cent total neutrophiles, it would be *impossible for any immature-mature ratio to indicate a fatal outcome.* Yet, in spite of this fact, and the fact that the significance of the immature-mature ratio depends almost wholly upon the percentage of total neutrophiles, the following may be found in a current textbook, frequently used by medical students and technicians:

IMMATURE		MATURE	SIGNIFICANCE
1	:	5	Lower limit of normal for mature forms.
1	:	3	Definite infection.
1	:	1	Severe infection.
1+	:	1	Frequently fatal, if persists.

It seems far better to us to use some standard, which remains the same under all conditions. The interpretation given by Osgood, which we have incorporated in our method for constructing the curve of the neutrophile changes, we consider much superior to the above. This is based upon the absolute increase in the number of staff cells, over 10 per cent indicating a severe infection; over 25 per cent, a very severe infection; and over 50 per cent a fatal outcome. It furnishes a fixed standard, which remains unchanged regardless of the fluctuations in the other factors in the differential count.

The slope of the line or curve is of great importance. A large number of immature neutrophiles has a much more serious import if the neutrophile percentage is greater than can be accounted for by the mere elevation of the total white count. This is also true if the increase in immature neutrophiles contains metamyelocytes and myelocytes. A "regenerative shift" is always of graver prognostic significance than a "degenerative shift."

Thus, by the use of the chart, one is enabled to grasp all the various complementary factors in the differential count at a glance. The presence and approximate amount of metamyelocytes and myelocytes, the degree of increase in immature neutrophiles, the percentage of total neutrophiles, and the extent of the patient's resistance in relation to all these factors, can all be portrayed in a few seconds by the construction of a simple curve; and, therefore, it is evident that an entirely different set of principles for appraising the prognosis from those which could be expressed by the original Gibson Chart can be laid down.

It is well to bear in mind the chief exceptions to the rules which have been made concerning increases in staff cells and metamyelocytes. Malaria is given as the chief exception to the rule that over 50 per cent staff cells indicates a fatal outcome. There is also a rule in regard to metamyelocytes, that the presence of more than 5 per cent in the blood indicates a seriously ill patient. The exceptions given are malaria, osteomyelitis, and smallpox. We are inclined to think that diseases ordinarily characterized by the presence of myelocytes and metamyelocytes in the blood, but without a very high mortality, of which the typhus fever seen in this country is a good example, also belong in this class. Schilling speaks of "the gay blood picture" which characterizes typhus, by which he means the leucocytosis, the high staff count, and the myelocytes and metamyelocytes, which are usually seen.

SUMMARY

A method for expressing the essential elements of the leucocyte count in the form of a curve is described. It presents the following new features:

1. It expresses the increase in immature neutrophiles (or "shift to the left"), both qualitatively and quantitatively, in addition to the total white cell count and neutrophile percentage.

2. When the method is applied to the interpretation of the leucocyte count in a number of pathologic states, various forms or "types" of curves result, which enables a given count to be at once placed within one of the different classes.

3. It simplifies the interpretation of the leucocyte count from the standpoint of prognosis.

4. It makes it possible to record the entire blood picture in the form of a chart during the course of an illness, showing the point at which metamyelocytes and myelocytes first appear in the blood, the rate and degree of increase in the total immature neutrophiles, and the extent of the patient's resistance in relation to the progress of the infection.

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AN ANALYSIS OF COLORIMETRIC METHODS IN RELATION TO PLASMA VOLUME DETERMINATIONS*

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A FEW years ago the author found that certain bodily states which are attended by thirst and a reduced salivary flow are also associated with a decrease in the plasma volume (Gregersen, 1932, Gregersen and Bullock, 1933). In the course of these experiments, however, it soon became evident that repeated determinations of plasma volume with the method devised by Keith, Rowntree and Geraghty (1915) were of questionable accuracy, even when the modifications suggested by Smith (1920) were followed. The author therefore decided to abandon the original investigation until a reliable technic for repeated determinations could be developed. With this purpose in mind the dye method has been examined in detail and the assumptions on which it is based have, so far as possible, been tested experimentally. The final reports have been withheld for nearly three years in order to try out the technic in a variety of physiologic problems; the experience gained has above all been valuable in demonstrating the limitations of any dilution method for estimating plasma volume. In view of the growing interest in the study of the water balance, it now seems desirable to report briefly on some of the more important questions which have been considered. The intention has not been merely to prepare a guide for those wishing to use the dye method but to provide a basis for evaluating the mass of blood volume data which has accumulated since Keith, Rowntree, and Geraghty introduced the plasma-dye method in 1915. Nevertheless, it is important to remember that so many conflicting results have been obtained with the plasma-dye method during the past twenty years that the testing of the method itself has in reality become the cardinal issue. First to be considered is the problem of colorimetry. The difficulties encountered and ways of overcoming them may be illustrated by discussing only three instruments, the simple colorimeter, the compensating colorimeter, and the spectrophotometer.

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The Simple Colorimeter.—The ordinary colorimeter is not satisfactory for accurate work unless the solvent itself is entirely clear and colorless. Such an ideal condition is, of course, never approached in plasma. To cancel the natural plasma color Keith, Rowntree, and Geraghty (1915) made up the standard and unknown in the same dilution of plasma constituents. Even so, it must be obvious that the contribution which the plasma itself makes to the total color effect is not the same in the standard and unknown solutions unless the solutions are the same depth. True cancellation of the natural plasma color is therefore not obtained unless the dye concentration happens to be exactly the same in both.

An examination of the literature reveals that these facts have not been universally recognized and it will be seen presently that a failure to do so may lead to serious errors, especially when the plasma contains a residuum of dye from a previous injection. Under this circumstance the plasma volume must be calculated from the *increase* in plasma-dye concentration resulting from the injection of a known quantity of a standard dye solution. Two methods for measuring this *increase* in dye concentration with the simple colorimeter have been worked out by Smith (1920; 1925), but each involves two separate colorimetric determinations and a number of laborious manipulations. Although Smith's methods do not seem to be practicable (Miller and Poindexter, 1932), his analysis of the problem makes it possible to account for some of the paradoxical and conflicting results which have been obtained with repeated determinations (Robertson and Bock, 1919; Lamson and Rosenthal, 1923).

The conditions which limit the use of the simple colorimeter and the errors incurred by ignoring them may be seen from the equation

$$\frac{R_2}{100} = \frac{R_1 + R_3}{R_1 + 100} \text{ (Smith, 1920).}$$

"R_2 = observed colorimetric reading (expressed in per cent) of a sample of dye-colored plasma taken after the injection of dye . . . . against a standard prepared from dye-tinged plasma taken immediately before injection of dye. . . . R_1 = the observed colorimeter reading of a sample of dye-tinged plasma . . . taken immediately before the injection of the dye . . . read against a standard prepared . . . (from) normal dye-free plasma. . . . R_3 represents the increase in concentration of dye in the plasma as a result of the injection." As Smith states, "this formula simply means that the unknown ($R_1 + R_3$) divided by the standard ($R_1 + 100$) equals the observed colorimetric reading in parts per 100 (i.e., R_2)."

From the above equation, we may conclude:

(1) $R_2 = R_3$ when $R_1 = 0$ (no residual dye).

(2) If $R_2 = 100$, then $R_3 = 100$ at any value of R_1 (i.e., when standard and unknown are the same strength no error results from the presence of residual dye).

(3) When $R_2 > 100$, $R_3 > R_2$ (i.e., when the standard is weaker than the unknown, the observed dye concentration is too low and the calculated plasma volume correspondingly high).

(4) Conversely, when $R_2 < 100$, $R_3 < R_2$ (i.e., when the standard is stronger than the unknown, the observed dye concentration is higher than the true value and the calculated volume accordingly too low).

(5) Any difference between R_2 and R_3 increases with R_1.

The degree to which residual dye (R_1) may affect the measurement of plasma volume when the observed colorimetric reading (R_2) is not equal to 100 per cent is clearly demonstrated by the following experiment.

The plasma volume of a normal dog was determined four times in about three hours. In the initial determination the concentration of the standard (1/300) was adjusted to equal that of the unknown ($R_2 = 100$ per cent). In the succeeding determinations four standards (1/200, 1/250, 1/300 and 1/400) were prepared each time from the dye-tinged control plasma obtained immediately before the injection of dye. It may be seen in Fig. 1 that except with the 1/300 standards against which the unknowns gave colorimetric readings of 100 per cent the results did not check with the original determination. The magnitude and direction of the error is obviously determined by the relative concentrations of the standard and unknown. If the standard is stronger than the unknown, the estimated volume is too low; if it is weaker than the unknown, the reverse is true. The range of error is apparently so large that extensive changes in plasma volume might readily be missed with such a method. It is hardly necessary to point out that lipemia and to a lesser degree

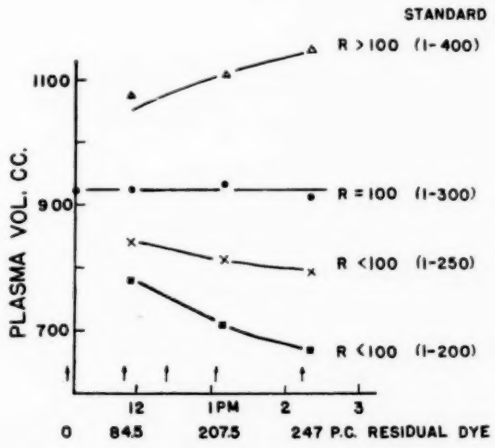

Fig. 1.—Repeated determinations of plasma volume with the Keith-Rowntree method showing the results obtained by reading each unknown against different standards. June 19, 1933. Dog 20.6 kg. Injections of 4 c.c. of 1.0 per cent brilliant vital red marked by arrows. The third determination was not completed due to hemolysis of the samples. For discussion of experiment see text.

the natural plasma color may also invalidate the results, and therefore the unknown should in every case be read against a standard which matches it very closely.

The Compensating Colorimeter.—Colorimeters that incorporate the principle of the compensating block comparator, well-known in connection with colorimetric pH determinations on tinted fluids such as urine or plasma (Clark, 1928), have in recent years been widely used for determinations of plasma volume (Seyderhelm and Lampe, 1925; Lindhard, 1926; Fleischer-Hansen, 1929-1930; Rusznyak, 1927; and others). A schematic diagram of one of these instruments, the Ellinger modification of the Bürker (1924) colorimeter is shown in Fig. 2. The cups are fixed, and the two plungers on each stage can only be moved together; hence the light beams *A* and *B* always traverse equal depths of fluid. With the arrangement of the solutions indicated in Fig. 2 the color contributed by the solvents is automatically cancelled, regardless of the

relative concentrations of dye in the standard and unknown and of other differences in their composition. For this reason a number of investigators have been led to simplify their technic by using dye standards prepared in water (Lindhard, 1926; Fleischer-Hansen, 1929, 1930; Griesbach, 1921, 1928; Schmidt, 1927; and others). Such a practice is not permissible with many vital dyes because their spectral absorption curves in water and plasma are quite different (Gregersen and Gibson, 1937).

The compensating colorimeter should also eliminate the difficulties with residual dye. If a portion of the dye in the unknown is residual, the same

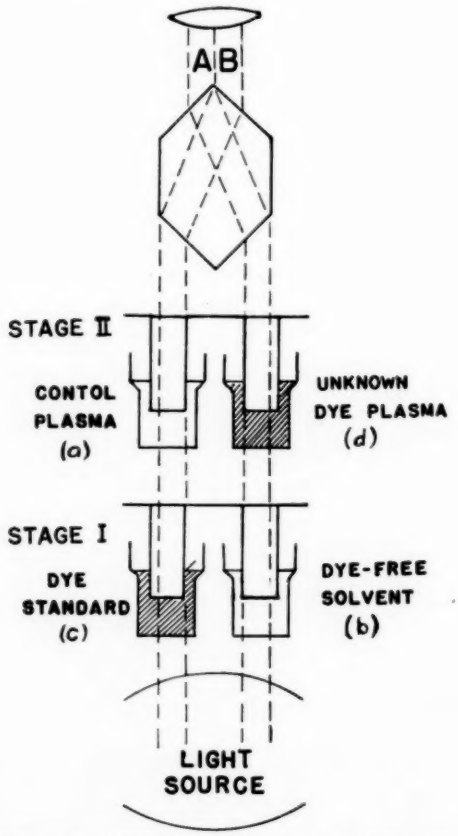

Fig. 2.—Diagram of the Ellinger modification of the Bürker colorimeter. (After Freund, 1932.)

concentration of residual dye will, of course, be present in the control sample. Under these conditions the dye standard in *a* (Fig. 2) is not compared with the total dye concentration in *d* but only with the difference in dye concentration between *c* and *d*, i.e., with the increase in color obtained as the result of the injection. Hence, a second or third determination of plasma volume should be no more complicated than the first in which the control plasma is actually free from dye.

In spite of its theoretical advantages, the compensating colorimeter proved to be very unsatisfactory. Most disturbing was the observation that in the analysis of a plasma dye sample the ratios obtained with the standard adjusted

to various depths sometimes showed differences of 30 to 40 per cent. The cause of this was not discovered. Furthermore, if the samples showed any lipemia the results were quite unpredictable, a fact also reported by Lindhard (1926). Known solutions were tested against themselves in an attempt to study the nature of the error caused by lipemia, but the only conclusion which could be drawn was that the readings fell nearer the correct values if comparisons were made with fluid layers 5 mm. or less in depth. Curiously enough, a series of known solutions of brilliant vital red made up in diluted milk gave correct readings although the scattering of light in these solutions was intense.

Practical difficulties appear also when the blood samples are diluted with isotonic oxalate to prevent clotting (Hooper, Smith, Belt and Whipple, 1920). Since the control and unknown must be identical in every respect except for the dye concentration, it is obvious that analysis with the Bürker colorimeter is not valid unless the plasma dilution is exactly the same in both samples. This is especially true when the plasma contains residual dye. Inasmuch as the oxalate must be added to the whole blood, the degree of dilution is always a matter of chance.

Finally, there is the particularly troublesome problem of hemolysis. The red dyes which have been commonly used for determining plasma volume (brilliant vital red, congo red, vital red, or trypan red) match the hemoglobin so closely that hemolysis of a dye-tinged sample may escape notice and give a totally erroneous reading for the concentration of the dye. The analysis is quite impossible if either the control or the unknown is hemolyzed. Consequently it is necessary to discard all samples which show even faint hemoglobin bands spectroscopically (Griesbach, 1921, 1928; Lindhard, 1926).

*The Spectrophotometer.**—This instrument offers a solution for the difficulties which are encountered with the colorimeters. Not only is the accuracy of measurement (1 per cent or less) far greater than with the colorimeters, but the determinations may be made on smaller samples (0.5 to 1.0 c.c.). Since the color is measured in absolute terms (optical density), the spectrophotometer eliminates the necessity of preparing standard solutions at the time of analysis of the unknown. Ordinarily the natural plasma color or residual dye is directly cancelled by reading the unknown against the control in a pair of identical absorption cells. If this is impossible due to unequal oxalate dilutions, the optical density of each sample can always be read separately, and appropriate corrections made for the differences in dilution.

Lipemia, which makes it impossible to employ the compensating colorimeter, does not interfere with the correct determination of the dye concentration with the spectrophotometer, provided the control shows the same degree of opalescence as the unknown. This was demonstrated in the following experiment: Two samples of plasma, one clear and the other showing intense lipemia (one hour after a fatty meal), were taken from the same dog and mixed in various proportions. Two-tenths of a cubic centimeter of a standard dye solution (1/50 of 1 per cent T-1824) were then added to 1.8 c.c.

*For description, theory and use of spectrophotometers the reader is referred to Clark (1928), H. P. Smith (1930) and also to "The Practice of Spectroscopy" by Twyman and Allsopp, (1934) which, although it deals with only one make of instrument, contains information that applies to all types of spectrophotometers.

of each mixture, while control (dye-free) solutions were prepared by adding 0.2 c.c. of saline to another 1.8 c.c. portion of each. When the samples were read in the spectrophotometer against their corresponding dye-free controls, the optical densities were found to be the same for all. The presence of fat does not, therefore, alter the optical density of the dye.

Nevertheless, lipemia cannot be ignored in carrying out plasma volume determinations. Any alteration in the intensity of the lipemia between the taking of the control sample and any subsequent dye sample will, of course, introduce an error in the determination. This is especially disturbing in studies on the disappearance rate of dyes from the blood stream, in which case it must be tacitly assumed that no change has occurred in the optical density of the plasma itself during the experiment. Lipemia can ordinarily be avoided by using only fasting animals or subjects, but in those cases in which it is an inescapable feature (e.g., myxedema), the plasma samples should be washed with a fat solvent before spectrophotometric analysis is attempted. Ethylene chloride is the best clearing agent tried so far, but it is not entirely satisfactory. After this treatment the samples are still somewhat opalescent.

The great advantage of spectrophotometric analysis is best seen in dealing with hemolysis. Whereas the error from this source cannot be avoided with a colorimeter, the spectrophotometer offers two possible ways of coping with the problem.

1. It is quite apparent from Fig. 3 that a simple spectrophotometric determination in plasma of a red dye such as brilliant vital red is out of the question if the sample is so much as slightly hemolyzed. Nevertheless, the spectral absorption curve of this dye differs sufficiently from that of hemoglobin to make possible a correction for hemolysis (Graff and Clarke, 1931). The method is in principle the same as that described by H. P. Smith (1930) for the analysis of a mixture of brilliant vital red and niagara sky blue, and depends upon readings taken at two points of the spectrum where one substance shows maximal and the other nearly minimal absorption. This procedure is time consuming and involved and, if the control sample also happens to be hemolyzed, it is impossible to determine accurately the absorption due to the natural plasma color. For routine experiments the method cannot be recommended.

2. Corrections for hemolysis become practically unnecessary by the choice of an appropriate dye. It may be seen from Fig. 3 that the spectral absorption of hemoglobin falls off sharply between 580 and 600 mu., and that in the region from 610 to 700 mu. it is relatively insignificant. Blue dyes which have maximal absorption in this part of the spectrum could, therefore, be determined directly in hemolyzed samples without much error; but the majority of blue dyes leave the blood stream so rapidly that they cannot be used for determining the plasma volume. However, among those studied by Dawson, Evans, and Whipple (1920) and by Gregersen, Gibson, and Stead (1935), there was one, T-1824, which disappeared from the plasma at a very slow rate (5 to 10 per cent per hour). Since the spectral absorption peak of T-1824 in plasma (Fig. 3) falls at 620 to 625 mu., a considerable degree of hemolysis

is required to interfere seriously with the direct determination of this dye. It should also be pointed out that the cancellation of the absorption due to the plasma itself assumes a less important rôle with blue than with red dyes, since the absorption curve of plasma is considerably lower at and beyond 600 mu. than it is at 500 mu.

Other investigators have recommended the use of blue dyes for plasma volume determinations (Dawson, Evans and Whipple, 1920; Harris, 1920; Seyderhelm and Lampe, 1922, and others) but only because blue seems rather easier to read in a colorimeter and does not obscure hemolysis. In my experience, however, a blue dye presents no great advantage unless the analysis is carried out with a spectrophotometer. In fact, it has the distinct disadvantage that the amount which must be injected (1 mg. per kilogram) to yield a

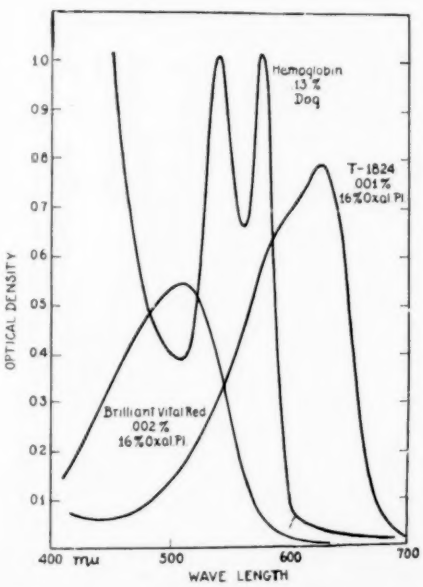

Fig. 3.—Spectral absorption curves (Hardy's self-recording spectrophotometer of brilliant vital red, hemoglobin and the blue dye T-1824, showing that spectrophotometric determinations of the latter in plasma are affected relatively little by the presence of moderate amounts of hemoglobin, whereas in the case of red dyes, such as brilliant vital red, hemolysis introduces a large error.

plasma concentration that can be read conveniently in a colorimeter causes marked staining of the skin and sclera and gives the subject an unhealthy, cyanotic appearance. It was for this reason that Seyderhelm and Lampe (1925) abandoned the trypan blue method which they had previously advocated (1922). If, on the other hand, the dye determinations are made with a spectrophotometer equipped with 20 mm. absorption cells it is possible, without sacrificing accuracy, to use such small doses of dye that staining is practically eliminated. T-1824, for example, can be measured accurately in concentrations ranging from 0.001 to 0.0005 per cent. Therefore, 20 mg. of this dye are sufficient for a plasma volume determination in man. This amount does not produce visible staining.

CONCLUSIONS

1. The use of the simple colorimeter for determining plasma volume with the dye method may involve gross errors, especially when the plasma contains residual dye (Smith, 1920). The result obtained depends very largely upon the relation between the strength of the standard and the unknown (Fig. 1).

2. The compensating colorimeter fails to yield dependable results under conditions which appear to be suitable for estimating the plasma-dye concentration with this type of instrument. Furthermore, if the samples are turbid (lipemia) or hemolyzed, the compensating colorimeter cannot be employed at all (Lindhard, 1926).

3. These difficulties can be overcome by using a spectrophotometer. With this instrument the absorption due to natural plasma color, lipemia or residual dye is directly cancelled by reading the unknown (dye sample) against the control (dye-free sample); a second or third determination may therefore be carried out as accurately and as readily as the initial one.

4. It is evident from the spectral absorption curves in Fig. 3 that the tedious corrections for hemolysis necessary with red dyes (Graff and Clarke, 1931) can be eliminated, except in grossly hemolyzed samples, by using the blue dye T-1824.

The author wishes especially to acknowledge his indebtedness to Dr. W. B. Cannon who provided the costly equipment required for this investigation. It is also a pleasure to thank Dr. H. M. Evans of the University of California for the supply of T-1824 with which many of the preliminary experiments were carried out, and Dr. A. C. Hardy of the Massachusetts Institute of Technology for the use of his self-recording spectrophotometer.

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CORRELATION OF HISTOLOGIC STRUCTURE WITH CLINICAL FEATURES*

Exemplified by Laboratory Reports on Goiters

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IT IS a commonplace for one and the same histologic section of an enlarged thyroid to show several kinds of change, whatever the clinical picture may be. In consequence, both surgeon and physician are likely to assign less importance to the laboratory report than they would if each type of thyroid enlargement carried its own distinctive picture. On the other hand, there may be no evident deviation from the normal structure at all, in which case the surgeon is not willing to conclude that his thyroidectomy was superfluous. This lack of correlation between histologic structure and clinical features has also naturally led to a distinctive nomenclature on the part of surgeon, physician, and pathologist. To some extent this could be obviated by greater attention to detail in the recording of the microscopic characters, for, if the various kinds of change found in a given specimen were listed, and at least a relative numerical value assigned to each, a tangible composite could be offered the clinician, from which he could recognize correspondence with his own observations.

The normal thyroid gland is of course a *tout ensemble* of a number of smaller functional units. These in turn are composed of various simpler components: cells, vessels, nerves, connective tissue. The diseased gland shows modifications of these, and in addition other features belonging to general pathology.

Since the standard textbooks on histology are necessary laboratory equipment, it is superfluous to do more than enumerate the following component elements in the thyroid acinus unit: (1) secretory cells, comprising some six varieties (including the so-called "parafollicular cells") in each of which one considers (a) mitochondria, (b) Golgi apparatus, (c) granules; (2) the colloid; (3) the capillaries and lymphatics; (4) the sympathetic nerve fibers; (5) the connective tissue elements common to any organ. In the first place according to the functional state of the gland, we note in the sections "*R*," the resting or involution phase of the acinus (acini or "vesicles" fairly large, the contained colloid staining fairly deeply with eosin, lining cells cubical or flattened), and/or "*H*," the hyperactive acinus (acini smaller; poorly staining colloid, lining cells tending to columnar form). The same symbol "*H*" may stand for "hypertrophy" and also for "hyperplasia," in which condition all the acini show hyperactivity up to an actual over production of cells. "*I*" denotes "islets" of the so-called parafollicular cells, external to the acinus itself.

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Second, in pathologic states we notice aggregations of lymphocytes "*l*," varying from a few cells in number to collections big enough to call "lymphomas"; "*h*" stands for haemorrhages, which may occur into or between the acini; "*f*" stands for the fibrous stroma when abnormal in amount; calc = calcification. We also allow for collections of other inflammatory cells, collections of secretory cells of parathyroid type, collections of secretory cells mimicking adrenal cortical cells; and we allow for degenerative changes, especially in the arteries. Each and all of these components may occur focally "*F*" (as exemplified by the macroscopic adenoma), or diffusely "*D*" through the sections.

Fig. 1. Fig. 2.

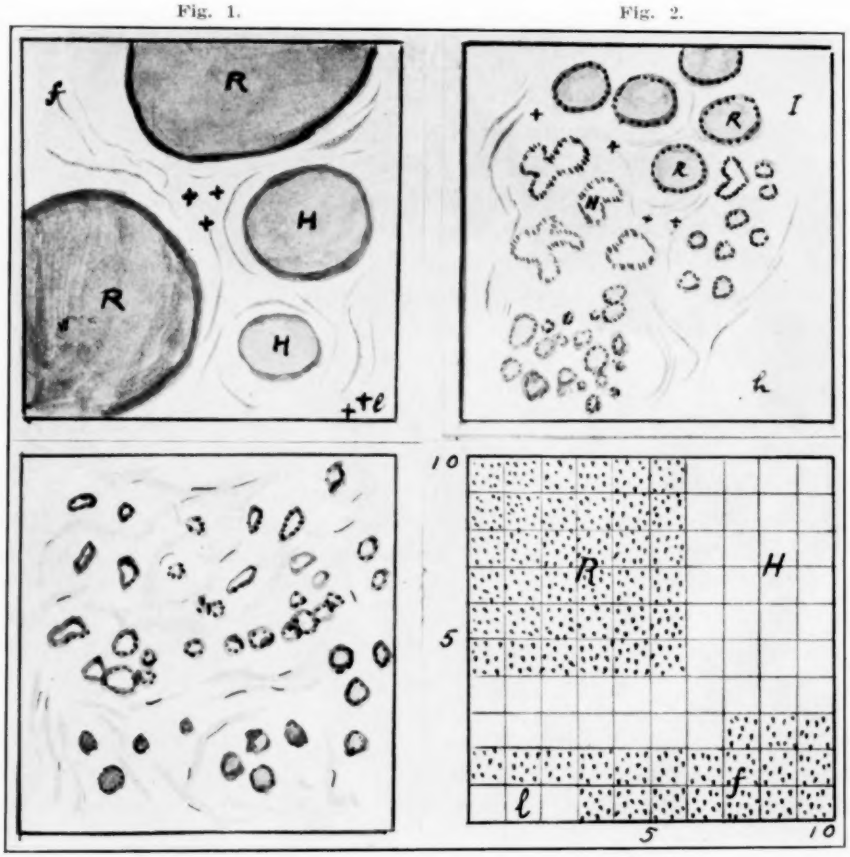

Fig. 3. Fig. 4.

PLATE I.—Figs. 1, 2, 3.—Interpretative sketches. Fig. 1.—Simple colloid, nontoxic goiter. Fig. 2.—Toxic goiter. Fig. 3.—Fetal adenoma.

The letters are the same as used in the text. In Fig. 1, the shaded areas represent either actual acini, or groups of similar acini, according to requirements. The + denotes lymphocytic infiltration. The two lighter areas denote collections of acini whose colloid contents stain less intensely. In Fig. 2, the hyperplastic acini are shown as having more elongated lining cells.

Fig. 4.—Alternative representation, in which the proportions of the different components are given in graphic form: 36 per cent (i.e. 36 squares) of the tissue is made up of acini with normal colloid; 20 per cent is formed of interstitial tissue; 3 per cent (in area of sections) is infiltrated with lymphocytes. The rest of the tissue shows acini with feebly-staining colloid. With this diagram it is easier to put side by side the histologic and clinical pictures of the given case.

Using the following simple list of varieties, we may therefore exemplify this method of analysis:

VARIETY OF GOITER	FORMULA
1. Simple	D R (± F)
2. Adenomatous	n F (H < R) + n′ D (R ± f)
3. Combination of 1 and 2	n F (H < R) + n′ D (xH ± yR + zf)
4. Fetal	n (F/D) H + n′ D R (xI + l, h, f,) ± calc
5. Cystic	F (R + f) + D R
6. Exophthalmic	D (x H) + y I + l + h + zf
7. Malignant	F H′ + D (R ± H + f)

In the case of paired letters, the first small letter of the pair (n,n′, x,x′, y,z) stands for an unspecified quantity, as in algebra. An attempt at exact numerical values could be made by making actual measurements of a projection of the section upon squared paper, counting out how many squares or parts of squares are covered by the different types of acinus, etc.; or even cutting the picture out and weighing the different parts. This refinement would be reasonable only if many sections from one case were subjected to it. For practical purposes a rough graph could be drawn up or an interpretative sketch made in which the spatial disposition of the various components is indicated.

The third example given would be translated thus: "there are focal collections or adenomata present, whose acini are predominantly in the resting phase; but there is in volume a greater amount of diffuse change. In the latter there are so many (n) acini in the resting phase, so many (y) in the hyperactive phase, and so much (z) fibrosis."

Needless to say, the use of an algebraic form is only suggested for the laboratory files, for possible future statistical application. But it is the basis of the pathologist's actual official report, a copy of which is also filed in the laboratory.

THE BEARING OF HISTOLOGY ON THE CLINICAL MANIFESTATIONS

It is not the goal of the pathologist simply to record with an approach to mathematical exactitude the composition of his specimen. He seeks to ascertain the meaning of the pictures he presents. It is true that the specification of the picture itself, or the determination of its exact meaning may not alter the surgeon's or physician's practice on the given case in hand. Yet it is not a mere academic pastime to seek the meaning of the histologic features. One wishes to understand the disease, so that in future it is possible to realize just exactly what has gone wrong to produce that particular thyroid enlargement in that particular patient. In one kind of case the function of the gland is normal. It is the rate of change which is accelerated, or slowed down excessively. In another case there is actual dysfunction and this may be interpreted in various ways. For example, on a biochemical basis, it is visualized in terms of iodine metabolism. On a colloidal chemical basis, the iodine per se is not taken as the cause of the symptoms or lack of symptoms, or of the staining effects in the tissue-section. It sees the iodine as merely holding the thyroid globulin in place, since lack of iodine caused the protein constituents of the secretion to pass from gel state to sol, this change being what is visible in the stained sections, and accounts for toxic symptoms when the extent of colloid change reaches a certain threshold. A seemingly trivial loss of iodine may thus set the full clinical picture of thyrotoxicosis going in a moment.

The nuances of the histologic picture can now fall into their place, and the apparent discrepancies between clinical symptoms and pathologic report are bridged over. For instance, patches of "R" in the section mean intermittent or periodic iodine deficiencies, the acini having been exhausted at some time, here and there. Diffuse "R" change, means that the iodine deficiency was prolonged. Large acini mean that iodine-supply has been restored, whether by administration of lugol, or spontaneously. Hyperplastic foci in the section mean lack of supply of iodine, which is being met by increased secretion on the part of the thyroid.

Islets have a similar significance. Patches of "*I*" infiltration mean the presence of a secretion perverted in quality. Fibrosis denotes scarring after previous attacks of hyperactivity. Calcification denotes precipitation of colloid into inorganic irreversible material.

Working backwards, it would be possible for the pathologist to point out in the section, or in his finished diagram, the relation of the particular symptoms of the case as being the result of such and such biochemical processes or regressions, remembering always that the various distinctive symptoms do not arise until the amount of change reaches a certain threshold and unless there is an imbalance in the past of the associated incretions.

TABLE I

	FOCAL				DIFFUSE		
	ADENOMATOUS		CYSTIC		DR.		DH.
	TOXIC	NONTOXIC	TOXIC	NONTOXIC	TOXIC	NONTOXIC	TOXIC
FREQUENCY	23%	15%	4%	2%	35%	11%	9%
Commonest age	30-45	30-45	early and late	30-45	14-20	30-45	14-20
History over 2 yr.	15%	6%	4%	15%	33%	8%	None
Symptoms: Pressure	+	+	−	+	+	+	−
Symptoms: Cardiac	+	−	+	+	+	−	+
Symptoms: Nervous	+	−	+	−	+	+	+
Symptoms: Exophthalmos	−	−	−	−	+	−	+
Postoperative trouble	66%	8%	1%	1%	13%	2%	4%

Table I may serve to indicate how an analysis of histologic results harmonizes with an analysis of the clinical findings in a small series of cases of goiter (studied at St. Paul's Hospital, Saskatoon, Sask.).

SUMMARY

The subject of goiter is taken as a convenient example for showing how complex tissue changes can be represented in the records of a surgical pathologic laboratory. A similar method can be utilized for other types of material (appendicitis, urologic, gynaecologic, etc.).

In the case of goiter, it may be thought that the achievement of exactitude in histologic analysis should be the point at which the pathologist should fade out of the picture, leaving the field to the surgeon and physician. However, this is really the point at which the true function of the pathologist should begin. Even were that point to be the limit of achievement allowed, it would still be true that by this method the pathologist is making for a truer insight into the real nature of the disease in some particular patient, to whose ultimate advantage the work is directed.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

RENAL FUNCTION, Tests for, Don, C. S. D. Brit. M. J. p. 54, July 10, 1937.

Renal function tests have been carried out in sixty-four cases in which it was thought that kidney disease might be present. The tests used were the blood urea and the urea clearance test, the maximum specific gravity test, and the phenolphthalein test. In the first group of thirty-three cases, some loss of renal function was demonstrated, and the patients comprising this group were on the whole more incapacitated and death was more common than among the second group of thirty-one cases, in which no loss of renal function was observed. Many of the patients were followed up after their discharge from the hospital. On clinical grounds alone some of those included in the first group because of loss of renal function might have been included in the second group without causing the least surprise. The reverse is equally true.

With pronounced renal failure, as indicated by a high blood urea, the urea clearance and the phenolphthalein tests showed marked depression of renal function, but this was not true of the maximum specific gravity. On two occasions it was normal, or nearly so, when the blood urea was over 100 mg. In both cases there was edema, and the patient was passing very little urine. The ability to secrete urine was at fault, but the ability to concentrate was normal. In some cases where renal function was apparently not nearly as severely impaired, this test seemed to be very delicate; the same may be said of the urea clearance test and the phenolphthalein test, each in some cases appearing to be the most sensitive. A surprising number of patients showed a lowered phenolphthalein excretion, but this may possibly be due to the fact that many had a high blood pressure. The lack of parallelism between the tests, especially in the lesser grades of renal damage, was most disappointing. None of them appear to be capable of enabling the clinician to give a very much more accurate prognosis than is possible from a knowledge of the blood urea alone. However, if any of the tests show marked loss of renal function in the absence of a raised blood urea, it would probably be best to give a guarded prognosis. A reexamination of the patient in three months might help. Clinical examination will often furnish very valuable information, and must still be regarded as of the foremost importance.

To conclude, the failure of the tests to agree closely with one another suggests either that they are not completely reliable or that different renal functions are being measured.

SCARLET FEVER, Immunization Against, Peterman, M. G. Am. J. Dis. Child. **54**: 90, 1937.

The author summarizes his brief against the present method as follows:

The bacteriologists and most clinicians do not accept a specific strain of the hemolytic streptococcus as the etiologic agent of scarlet fever.

The bacteriologists insist that it is not possible to produce an active immunity against any streptococcus.

The specificity of the Dick test has not been proved.

Active immunization with the Dick toxin or vaccine prevents the development of a rash for two or more years. If the inoculated person becomes infected with scarlet fever, his physician is robbed of his most valuable diagnostic sign.

The Committee on Clinical Investigation and Scientific Research of the American Academy of Pediatrics reported on June 11, 1934: "The whole matter of scarlet fever immunization, both by the toxin and by the toxoid methods, is being investigated . . . by the U. S. Public Health Service and the Massachusetts State Board of Health. The results of these investigations must be awaited before it is possible to evaluate their usefulness." In April, 1935, the Special Committee on Prophylactic Procedures Against Communicable Diseases of the American Academy of Pediatrics reported: "Active immunization (against scarlet fever) . . . is not recommended as a general public health procedure, since reactions both local and general are frequent . . . and for the reason that the degree and duration of immunity have not been definitely established."

PREGNANCY, The Chemical Diagnosis of Early, Patterson, J. Brit. M. J., p. 522, September 11, 1937.

A biochemical test for the diagnosis of early pregnancy is described which is based upon bacterial splitting of estriol glycuronide and the subsequent development of the estriol color reaction with phenolsulphonic acid.

Urines from sixty-five cases in which pregnancy diagnosis was required have been examined by the test. In all except one case the result was in agreement with that of the Friedman reaction. The advantages and disadvantages of the test as compared with the biological tests are briefly discussed. The technique follows:

Urine Sample: The requirement of the test is a sample of at least 50 c.c. of early morning concentrated urine, collected into a vessel chemically clean in the sense of being entirely free from all substances which might be inhibitory to bacterial action. It is also advisable that the patient should not have had any drugs for a few days before the collection, for these often increase the pigmentation of the urine, and may also adversely affect the preliminary bacterial hydrolysis.

Preliminary Treatment: The urine is tested for high acidity by treating a few drops with methyl red, and if acid to this indicator, it is adjusted with addition of alkali until it no longer shows a pink color. A 50 c.c. sample is then heavily inoculated with *B. coli* and incubated overnight. To the urine after incubation, while it is still warm, is added approximately 0.5 gram of sodium bisulphite. The specimen is shaken until the solid has completely dissolved and then allowed to stand for fifteen minutes.

Extraction: The partially decolorized urine is transferred to a separatory funnel of about 150 c.c. capacity and extracted with two lots of 40 c.c. of ether. Occasionally some emulsification occurs, but this may be rapidly resolved by centrifugation. The combined ether extracts are washed with a little water, and then well shaken with a 30 c.c. portion of 10 per cent sodium carbonate. The alkali layer having been discarded, this washing process is repeated until the carbonate layer is completely colorless. The ethereal solution is then again washed with distilled water, and when the water layer has been drawn off, the ether layer is further extracted with two lots of 40 c.c. N/10 sodium hydroxide. After separation and rejection of the upper ethereal solution, the combined alkali portions containing the estriol are treated with 25 per cent sulphuric acid drop by drop until acid to Congo red paper. This acidified aqueous solution is then extracted with two portions of 40 c.c. of pure "analytical" ether, after which the ether layers are combined and washed with a little water. The ether extract is given another washing with 10 per cent sodium carbonate, and after the rejection of the alkali, the remaining ether is freed from all trace of the alkaline carbonate by two more washings with distilled water. The final clear, colorless ether solution is next transferred in two portions to a 50 c.c. transparent silica flask, and the solvent completely evaporated by immersing the flask in a large beaker of water previously heated to 70° C.; the last traces of moisture are then removed in vacuo by direct application of suction to the flask. The dry residue is now ready for the development of the color reaction, using the phenolsulphonic reagent.

Reagent: The reagent used is that recommended by Cohen and Marrian, and consists of 3.6 parts of pure phenol with 5.6 parts of pure concentrated sulphuric acid. Small quantities only are prepared at a time; 9 c.c. of phenol, liquefied by heating, to which are added 14 c.c. of sulphuric acid with sufficient external cooling to the container to prevent undue rise of temperature, makes a convenient amount. It can then be stored in a 25 c.c. all-glass buret, fitted with a ground glass stopper so as to exclude moisture from the reagent. Considerable care must be taken with the reagent, which is very hygroscopic and becomes inactive when it takes up water. In measuring out, therefore, it is important first to discard a volume equal to that portion in the tip of the buret below the stopcock, so that only completely protected reagent is used for the reaction. Moreover, it is advisable only to use a reagent which is less than one week old.

Color Reaction: To the flask containing the dry residue, 1 c.c. of reagent is added from the buret, and the flask then immersed in a large water-bath, previously heated to about 70° C. By frequently rotating the flask, the whole of the residue distributed around the sides is brought into the reagent, and the temperature of the bath is rapidly raised to boiling point and kept there for a period of exactly ten minutes. During the boiling period, it is again advisable from time to time to rotate the reagent around the sides of the flask. The reagent at this stage has assumed a yellowish color, and it is now cooled by holding the flask under a stream of tap water. While this cooling process is going on, 1 c.c. of 5 per cent sulphuric acid is slowly added, the contents of the flask being kept moving in order to bring the somewhat syrupy reagent into a homogeneous solution with the dilute acid. The product, which is still yellowish, is then reheated in the boiling water-bath for a period of two and a half minutes. A positive reaction is obtained when the original color gradually changes over to pink or red; a negative reaction, when this change is entirely absent.

MONONUCLEOSIS, Infectious, Diagnostic Value of Supravital Staining in, Gall, E. A. Am. J. M. Sc. **194**: 546, 1937.

There is fairly generally admitted difficulty in definitely identifying the specific cell of infectious mononucleosis.

Despite the evident value of the determination of the presence of heterophile agglutinins, recognition of characteristic morphologic features of the typical cell of the disease would obviate its use to a great extent. Such features are evident in supravitally stained preparations.

By this means the cell is definitely shown to be an atypical but relatively mature lymphocyte, readily distinguished from other mononuclear cells by several characteristics.

Refractile granules present in one-third of the lymphocytes in other diseases and in the normal state are never present in higher than 15 per cent of the lymphocytes in infectious mononucleosis.

ANTHRACOSILICOSIS, Carcinoma of Bronchus in Association with, Charr, R. Am. J. M. Sc. **194**: 535, 1937.

Clinical and pathologic observations on 4 cases of primary carcinoma of the bronchus associated with anthracosilicosis are briefly described.

Dyspnea, hemoptysis, cough, expectoration, and pain in the chest were the outstanding symptoms. Sputum negative for tubercle bacilli (with hemoptysis a frequent symptom) excited a suspicion of primary malignant growth of the bronchus.

Roentgen ray diagnosis of diffuse bronchiogenic carcinomatosis is difficult when anthracosilicosis coexists.

Extensive destruction of the bronchial cartilages, resulting in collapse of the involved bronchi, might have been responsible for severity of dyspnea.

Capability of the peribronchial and perivascular lymphatics to dilate in spite of fibrosis surrounding them was noted.

Spread of tumor cells through the alveolar pores and interlobar adhesions was observed.

Absence of toxic symptoms of pulmonary suppuration was attributed to impaired lymphatic absorption and lessened blood flow through the atelectatic lungs.

A suggestion is made that in these cases anthracosilicosis might have had an indirect influence on development of carcinoma of the bronchus.

CHANCROIDAL TEST, Diagnostic Value of the Intradermal, Greenblatt, R. S., and Sanderson, E. S. Arch. Dermat. & Syph. 36: 485, 1937.

A total of 721 intradermal chancroidal tests were performed upon 191 patients, and conclusions are drawn as to the diagnostic value of the test.

The superiority of the chancroidal test with bacillary antigen, as advocated over the test with antigen prepared from the pus from buboes as described by Cole and Levin, is evaluated.

A warning note is sounded to investigators who report cases of lymphogranuloma venereum on the basis of a positive Frei test without performing a concurrent chancroidal test. Twenty-four patients in a group of 80 patients exhibiting a positive reaction to the chancroidal test also had a positive reaction to the Frei test; this emphasized the need for caution in the interpretation of the results of these tests.

A positive chancroidal test is only presumptive evidence and does not necessarily mean that the lesion under consideration is chancroidal. A similar conclusion should be borne in mind in the interpretation of a positive Wassermann as well as of a positive Frei test in every case of genital lesion, unless other confirmatory laboratory evidences are present.

Finally, a negative intradermal test with bacillary antigen is diagnostic so far as it rules out infection by the bacillus of Ducrey. A positive intradermal chancroidal test commits one to the diagnosis of chancroidal infection only when the Frei test, the Wassermann test, and the microscopic examinations for malignant disease and granuloma inguinale give negative results.

TRICHINOSIS, Studies On, Hall, M. C., and Collins, B. J. Public Health Reports, 1937.

Hall and Collins report upon a study of trichinosis conducted under the auspices of the National Institute of Health, United States Public Health Service.

A study of 300 diaphragms from cadavers, coming from 10 hospitals in Washington, D. C., and 1 hospital at Baltimore, Md., shows 41 diaphragms infested with trichinae, an incidence of 13.67 per cent.

The samples include cases from 5 Federal hospitals to which patients are sent from all over the United States, and from 6 Washington hospitals with cases originating widely over the United States. The cases run the range of childhood to old age, military and civil life, association with land and sea, sane individuals and mentally deranged hospitalized cases, black and white, male and female, and high and low economic-social status.

All diaphragms were examined by both the direct microscopic method and by the digestion-Baermann method, since both methods have special value for certain types of infestation and both have certain limitations, the two methods being supplementary in these respects.

On the basis of 1,778 cases reported up to the present time, the writers conclude that an indicated incidence of approximately 12.5 per cent, an unweighted average, is a conservative figure, probably definitely too low. If this figure is indicative of incidence throughout this country, there are probably several million persons in the United States who are infested with trichinae, among whom are possibly several hundred thousand who have had clinical trichinosis never diagnosed as such, and there are possibly several thousand deaths annually from this cause.

The following point is emphasized: The United States apparently has the greatest problem of trichinosis of any country in the world, a problem involving, in one way or another and in some degree, several million persons. The incidence in man is greater than the incidence in garbage-fed hogs.

A consideration of the age incidence by decades indicates that, at least up to some unascertained point, there is an increased incidence with increasing age, due apparently to the

fact that an increase in the time factor increases the opportunities for infection. At some unascertained point there may be a mortality factor in the form of deaths occurring at an age earlier than would have been the case had it not been for pathologic conditions persisting after recovery from trichinosis, thereby removing from the older age groups some of the positives that might otherwise have appeared there. Positive findings in recent literature indicate the advisability of examining suitable muscle tissue from very young infants and those prematurely born, for the possibility of detecting prenatal infection with trichinae. The postmortem study of suitable muscles from persons dying after prolonged confinement in hospitals, jails, and penitentiaries, under modern sanitary conditions precluding the eating of raw or undercooked pork, is suggested as a basis for obtaining more precise information as to the time larval trichinae survive alive in human beings, and the time required for calcification of the cysts and for the death of the trichinae. It is recommended that the microscopic examination of 1 gram of diaphragm muscle, as a press preparation and not by sectioning, be made a routine procedure in the postmortem examinations by pathologists.

Since examinations of 1,778 cadavers at 24 hospitals in 11 places in the United States indicate an incidence of at least 12.5 per cent of trichinae, with not 1 case out of 222 positive cases having been diagnosed as trichinosis at any time, it is evident that knowledge of the polymorphic picture of clinical trichinosis is inadequate and that we need more information in regard to diagnosis.

To clarify the picture of trichinosis, especially of atypical clinical cases caused by infestations of intermediate extent, as opposed to very heavy or very light infestations, calls for much research and cooperation by pathologists, clinicians, and parasitologists. Quantitative studies are especially necessary.

Garbage-fed swine have trichinae between three and five times as frequently as do grain-fed swine, and hence are especially important as sources of human trichinosis.

Trichinosis in swine is apparently traceable to the eating of uncooked pork scraps in garbage, table scraps, swill, and similar things, much more often than it is traceable to the eating of rats by swine.

The garbage-feeding industry, as ordinarily carried on, is dangerous to the health of man and livestock, is esthetically objectionable, and is often economically unsound.

Suggestions are made for the elimination of the dangers and nuisances associated with the garbage-feeding industry and with the feeding of table scraps and similar things on the farm. Cooperation between scientists, practicing physicians, engineers, packers, and the swine industry is recommended as the best attack on the problem.

The authors are emphatic in recommending the examination of excised tissue as a press preparation rather than by section, regarding the former method as not only more rapid but more accurate. They emphasize, also, the need for development of better methods of early diagnosis, especially in the atypical case.

PNEUMONIA DUE TO PNEUMOCOCCUS TYPE VII, Bullowa, J. G. M., and Greenbaum, E. Arch. Int. Med. **60**: 180, 1937.

It appears as the result of these studies that among the patients who received serum, the mortality was significantly reduced and the duration of illness was shortened, even when serum treatment was begun on the fifth, sixth, or seventh day of illness.

The patients to whom serum was administered before the fifth day of illness had no complications. For the group of patients who received serum on the fifth day or later and for the group who were not given serum, the incidence of complications was approximately the same, but the mortality among those with complications who were treated on the fifth day or later was lower than the mortality among the corresponding group of patients who did not receive serum treatment.

STAPHYLOCOCCIC IMMUNITY, Rigdon, R. H. Arch. Path. **24**: 233, 1937.

In this review of staphylococcic immunity, it has been found that vaccines have very little therapeutic effect on staphylococcic infections either experimentally or clinically. It is suggested, however, that they may stimulate the formation of antibodies in man.

Toxoid appears to be a very efficient antigen in the treatment of certain staphylococcic infections of low virulence in the skin and may be of some value in certain cases of chronic infections, such as osteomyelitis. Toxoid produces an increase in the circulating antihemolysins, and this is frequently accompanied by clinical improvement.

The experimental and clinical results suggest that staphylococcus antitoxin has a definite therapeutic value in treatment in certain cases of acute staphylococcic infections which are accompanied by toxemia. This antitoxin may act in the following ways: The toxin produced by the staphylococcus in vivo is neutralized by this antitoxin; a component of the immune serum may act on the staphylococci and increase the ease with which they are phagocytosed by certain organs, especially the liver and spleen. The antitoxin in the circulation may also inhibit the action of leucocidin and thus allow the polymorphonuclear leucocytes to phagocytose the bacteria in either the blood or the tissue.

From the experimental observations on the mechanism of immunity to staphylococcic infections, it appears that vaccine, toxin, or toxoid may also cause proliferation of the lymphoid and histiocytic elements, especially in the liver and spleen. This cellular change enhances the ability of these tissues to phagocytose the staphylococci.

MYXEDEMA, Anemia of: Classification and Treatment, Sharpe, J. C., J. A. M. A. 194: 382, 1937.

The exact etiology of the anemia of myxedema is unknown, but it is probably the result of decreased blood formation, due to the hypometabolic effect on the bone marrow.

In addition to the usual physical signs of myxedema, the anemic patient may present such clinical features as a lemon yellow pallor, glossitis, paresthesias, and an achlorhydria. An erroneous diagnosis of pernicious anemia may be made.

In 3 cases, the mean corpuscular volume and the hemoglobin content of the red blood cells showed that the anemia was normocytic and normochromic in character. In the fourth case an achlorhydria, hypochromia, and microcytosis may have been due to an associated iron-deficiency anemia.

Though thyroid extract causes a prompt increase in the basal metabolic rate, a transient decrease in the blood count may first be noted, followed by a slow and sustained rise in both hemoglobin and erythrocytes.

In the presence of hypochromia, iron may accelerate the regeneration of blood cells, but liver extract appears to be of no value in this type of anemia.

HYPERTENSION, Arterial, Site and Significance of High Chloride Content of Blood, Apperly, F. L., and Cary, M. K. Am. J. M. Sc. 194: 352, 1937.

In a study to determine the site of increased blood chloride in patients with arterial hypertension, and if possible its significance, it was found:

1. The increased blood chloride is wholly confined to the red cells.

2. This increased cell chloride is not the result of acidemia, since pH and erythrocytic volume-index showed no significant deviations from the normal.